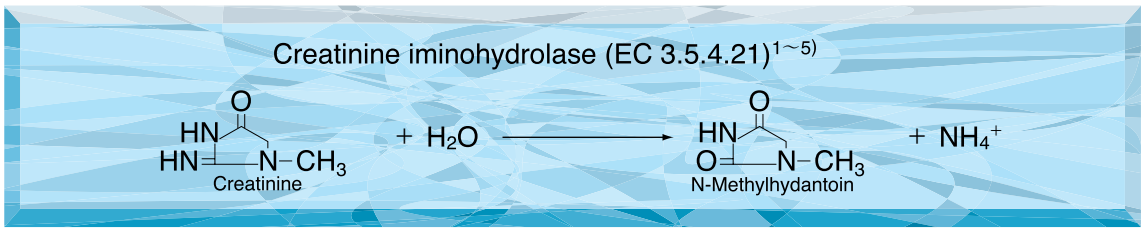
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

CREATININE DEIMINASE

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized	
Activity	: Grade Ⅲ 10U/mg-solid or more	
	(containing approx. 30% of stabilizer)	
Contaminants	: Creatinine amidohydrolase	≤1.0×10^{-2}%
	Creatine amidinohydrolase	≤1.0×10^{-2}%
	Urease	≤1.0×10^{-2}%
	NADH oxidase	≤1.0×10^{-2}%
	NH_4^+	≤1.0×10^{-2} μg/u
Stabilizer	: Mannitol	

PROPERTIES

Stability	: Stable at −20℃ for at least 6 months	(Fig.1)
Molecular weight	: approx. 260,000	
Isoelectric point	: 4.4	
Michaelis constant	: 3.5×10^{-3}M (Creatinine)	
Structure	: 6 subunits per mol of enzyme	
Inhibitors	: Ag^+,Hg^{++}, o-phenanthroline,monoiodoacetate	
Optimum pH	: 8.5−9.5	(Fig.2)
Optimum temperature	: 65−75℃	(Fig.3)
pH Stability	: pH 7.0−11.0 (30℃, 20hr)	(Fig.4)
Thermal stability	: below 65℃ (pH 7.5, 1hr)	(Fig.5)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of creatinine when coupled with glutamate dehydrogenase (GTD-211, GTD-209, GTD-309) in clinical analysis.

ASSAY

Principle:

Creatinine + H_2O + H^+ $\xrightarrow{\text{creatinine deiminase}}$ N-Methylhydantoin + NH_4^+

NH_4^+ + α-Ketoglutarate + NADPH $\xrightarrow{\text{GIDH}}$ Glutamate + H_2O + $NADP^+$

GIDH:Glutamate dehydrogenase($NADP^+$ dependent) [L-Glutamate:NADP oxidoreductase (deaminating), (EC 1.4.1.4.)]

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

Unit definition:

One unit causes the formation of one micromole of ammonia (the oxidation of micromole of NADPH) per minute under the conditions described below.

Method:

Reagents

A. Creatinine solution : 50mM (565.5mg creatinine/100ml of 50mM K-phosphate buffer, pH7.5)

B. NADPH solution : 3.0mM (27.2mg NADPH・Na_4・$4H_2O$/10ml of 50mM K-phosphate buffer, pH 7.5)(Should be prepared fresh)

C. α-Ketoglutarate solution : 10mM (14.6mg α-Ketoglutaric acid/10ml of 50mM K-phosphate buffer, pH 7.5)(Should be prepared fresh)

D. GIDH solution : ca.1,000U/ml [Dilute Toyobo Grade Ⅱ (Tris-HCl buffer solution, free from ammonia to ca.1,000U/ml with H_2O.)]

E. Enzyme diluent : 50mM K-phosphate buffer, pH 7.5

Procedure

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

2.4 ml	Substrate solution	(A)
0.3 ml	NADPH solution	(B)
0.3 ml	α-Ketoglutarate solution	(C)
0.05ml	GIDH solution	(D)

Concentration in assay mixture	
K-Phosphate buffer	49 mM
Creatinine	38 mM
α-Ketoglutarate	0.95mM
NADPH	0.29mM
GIDH	ca.16 u/ml

2. Add 0.10ml of the enzyme solution* and mix by gentle inversion.
3. Record the decrease in optical density at 340nm against water for 3～4 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 by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution (ΔOD blank).

* Dissolve the enzyme preparation in ice-cold enzyme diluent (E) and dilute to 0.15－0.4U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min (}\Delta\text{OD test} - \Delta\text{OD blank)} \times \text{Vt} \times \text{df}}{6.22 \times 1.0 \times \text{Vs}} = \Delta\text{OD/min} \times 5.06 \times \text{df}$$

Weight activity (U/mg)=(U/ml)×1/C

Vt : Total volume (3.15ml)

Vs : Sample volume (0.10ml)

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

1.0 : Light path length (cm)

df : Dilution factor

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

REFERENCES

1) J.Szulmajster; *Biochim.Biophys.Acta, 30,* 154 (1958).

2) T.Uwajima and O.Terada; *Agr.Biol.Chem., 40,* 1055 (1976).

3) T.Uwajima and O.Terada; *Agr.Biol.Chem., 41,* 339 (1977).

4) D.Tsuru; *Rinsho Kensa, 22,* 1331 (1978).

5) T.W.Esders and S.Y.Lynn; *J.Biol.Chem., 260,* 3915 (1985).

Table 1. Effect of Various Chemicals on Creatinine deiminase

[The enzyme dissolved in 50mM Tris-HCl buffer, pH 7.5 (2U/ml) was incubated at 25℃ for 2hr with each chemical. The residual activity was assayed according to the routine method described above.]

Chemical	Concn.(mM)	Residual activity
None	–	100%
Metal salt	1.0	
$MgCl_2$		103.8
$CaCl_2$		100.6
$Ba(OAc)_2$		105.7
$FeCl_3$		5.0
$CoCl_2$		108.2
$MnCl_2$		179.0
$ZnSO_4$		100.0
$Cd(OAc)_2$		143.0
$NiCl_2$		103.2
$CuSO_4$		2.1
$Pb(OAc)_2$		87.3
$AgNO_3$		1.1
$HgCl_2$		1.6
2-Mercaptoethanol	1.0	96.8
PCMB	0.1	91.1
MIA	1.0	0.6
NEM	1.0	86.1
IAA	1.0	60.1
Hydroxylamine	1.0	86.1
EDTA	2.0	96.2
o-Phenanthroline	1.0	0.7
α,α′-Dipyridyl	0.1	100.6
Borate	5.0	98.7
NaF	1.0	100.6
NaN_3	1.0	99.4
TritonX-100	1.0%	95.6
Brij 35	0.1%	83.5
Span 20	0.5%	103.2
Na-Cholate	0.5%	100.0
SDS	0.5%	101.0
DAC	0.5%	81.8

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

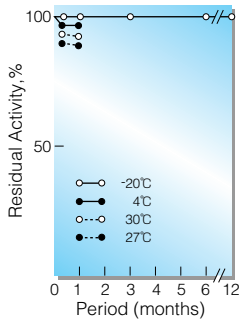


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

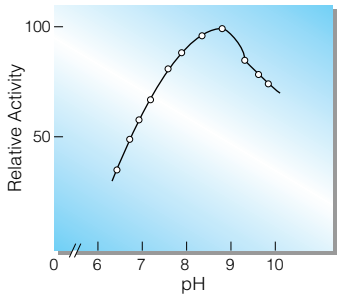


Fig.2. pH-Activity

〔37℃ in Britton-Robinson buffer; The activity was assayed by the indophenol method.[3]〕

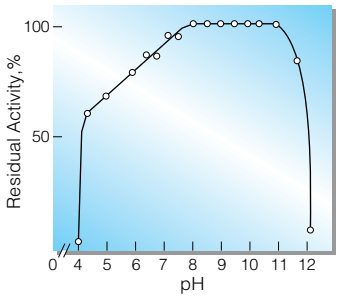


Fig.4. pH-Stability

〔30℃,20hr-treatment with Britton-Robinson buffer〕

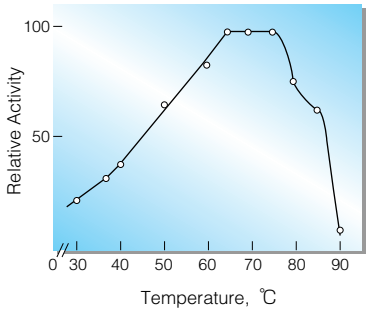


Fig.3.Temperature activity

〔in 50mM K-phosphate buffer, pH 7.5; The acitivity was assayed by the indophenol method.[3]〕

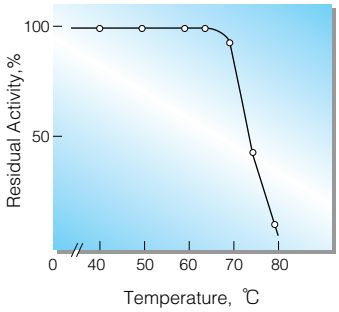


Fig.5. Thermal stability

〔1hr-treatment with 50mM K-phosphate buffer, pH7.5〕

活性測定法（Japanese）

1.原理

Creatinine＋H_2O＋H^+ $\xrightarrow{\text{creatinine deiminase}}$ N-Methylhydantoin＋NH_4^+

NH_4^+＋α-Ketoglutarate＋NADPH $\xrightarrow{\text{GIDH}}$ Glutamate＋H_2O＋$NADP^+$

NADPHの消失量を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1マイクロモルのアンモニアを生成する酵素量を1単位 (U)とする。

3.試薬

A. 50mMクレアチニン溶液(565.5㎎のクレアチニンを50mM K-phosphate buffer, pH7.5 100㎖に溶解する)(用時調製)

B. 3.0mM NADPH溶液(27.2㎎ NADPH・Na_4・$4H_2O$を50mM K-phosphate buffer, pH7.5 10㎖に溶解する)(用時調製)

C. 10mM α-ケトグルタル酸溶液 (14.6㎎のα-ケトグルタル酸を50mM K-phosphate buffer, pH7.5 10㎖に溶解する)

D. グルタミン酸脱水素酵素(GIDH)溶液〔Tris-HCl緩衝液(アンモニアを含有しない東洋紡製GIDHは本活性測定に適す)を約1,000 U/㎖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50mM K-phosphate buffer, pH7.5で溶解し,分析直前に同緩衝液で0.15－0.4U/㎖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,37℃で約5分間予備加温する。

2.4 ㎖	基質溶液	(A)
0.3 ㎖	NADPH溶液	(B)
0.3 ㎖	α-ケトグルタル酸溶液	(C)
0.05㎖	GIDH溶液	

②酵素溶液0.1㎖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分間あた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(50mM K-phosphate buffer, pH 7.5)を0.1㎖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

$$U/㎖ = \frac{\Delta OD/min\ (\Delta OD\ test - \Delta OD\ blank) \times 3.15(㎖) \times 希釈倍率}{6.22 \times 1.0 \times 0.10(㎖)}$$

$$= \Delta OD/min \times 5.06 \times 希釈倍率$$

U/mg ＝ U/㎖×1 / C

6.22 ： NADPH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/㎖)