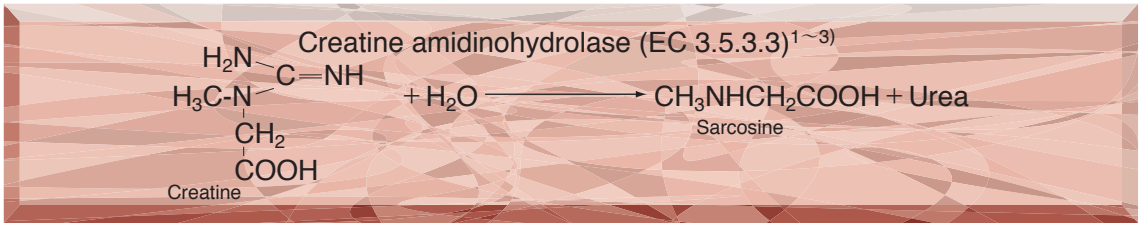
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

CREATINE AMIDINOHYDROLASE

from Actinobacillus sp.



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ 6.0U/mg-solid or more (containing approx. 50% of stabilizers)
Contaminants	: NADH oxidase ≤5.0×10^{-2}%
	Catalase ≤2.0%
Stabilizers	: Sugars, EDTA

PROPERTIES

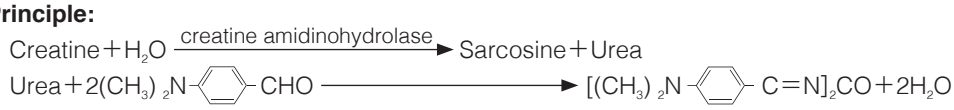
Stability	: Stable at −20℃ for at least one year	(Fig.1,2)
Molecular weight	: approx. 100,000	
Isoelectric point	: 4.6±0.1	
Michaelis constant	: 1.9×10^{-2}M (Creatine)	
Structure	: 2 subunits per enzyme molecule	
Inhibitors	: Cu^{++}, Hg^{++}, Ag^{+}	
Optimum pH	: 8.0	(Fig.4)
Optimum temperature	: 40℃	(Fig.5)
pH Stability	: pH 5.5−9.0 (25℃, 16hr)	(Fig.6)
Thermal stability	: below 50℃ (pH 7.5, 30min)	(Fig.7)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of creatine and creatinine when coupled with creatinine amidohydrolase (CNH-211, CNH-311) and sarcosine oxidase (SAO-351) in clinical analysis.[4]

ASSAY

Principle:



The appearance of yellow dye formed by condensation of urea and p-dimethylaminobenzaldehyde (DAB) (Ehrlich reaction) is measured at 435nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of yellow dye per minute under the conditions described below.

Method:

Reagents

A. Creatine solution : 0.1M [1.49g creatine (Nacalai tesque)/100ml of 50mM phosphate buffer, pH 7.5] (Should be prepared fresh).

B. DAB solution : Dissolve 2.0g of DAB in 100ml of dimethylsulfoxide and, to this solution, add 15ml of conc. HCl solution.

C. Enzyme diluent : 50mM Phosphate buffer, pH 7.5

Procedure

1. Pipette 1.0ml of the substrate solution (A) into a test tube and equilibrate at 37℃ for about 5 minutes.
2. Add 0.1ml of the enzyme solution* and mix.
3. After exactly 10 minutes at 37℃, add 2.0ml of DAB solution (B) to stop the reaction.
4. Incubate at 25℃ for 20 minutes.
5. Measure the optical density at 435nm against water (OD test).

   At the same time, prepare the blank by first mixing the substrate solution with 2.0ml of DAB solution after a 10 min-incubation at 37℃, followed by the addition of the enzyme solution, and carry out the same procedure as test (procedure 4 and 5)(OD blank).

Concentration in assay mixture	
Phosphate buffer	50mM
Creatine	90mM

* Dissolve the enzyme preparation in ice-cold enzyme diluent (C) and dilute to 2.0−3.0 U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD(OD\ test - OD\ blank) \times Vt \times df}{0.321 \times 1.0 \times t \times Vs} = \Delta OD \times 9.65 \times df$$

Weight activity (U/mg) = (U/ml)×1/C

Vt : Total volume (3.1ml)

Vs : Sample volume (0.1ml)

0.321 : Millimolar extinction coefficient of yellow dye (㎠/micromole)

1.0 : Light path length (cm)

t : Reaction time (10 minutes)

df : Dilution factor

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

REFERENCES

1) D.Tsuru; *Nucleic Acid and Amino Acids, 35,* 31 (1977).

2) T.Yoshimoto, I.Oka and D.Tsuru; *Arch.Biochem.Biophys., 177,* 508 (1976).

3) T.Yoshimoto ,I.Oka and D.Tsuru; *J.Biochem., 79,* 1381 (1976).

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

Table 1. Effect of Various Chemicals on Creatine amidinohydrolase

[The enzyme dissolved in 50mM Tris-HCl buffer, pH 7.5 (80U/ml) was incubated at 25℃ for 30 minutes with each chemical.]

Chemical	Concn.(mM)	Residual activity(%)
None	—	100
Metal salt	1.0	
$CaCl_2$		107
$MnCl_2$		109
$MgCl_2$		103
$NiCl_2$		107
$CoCl_2$		108
$Ba(OAc)_2$		104
$Cd(OAc)_2$		88
$FeCl_3$		103
$FeSO_4$		102
$HgCl_2$		2.7
$ZnSO_4$		97
$CuSO_4$		11
$Pb(OAc)_2$		108
$AgNO_3$		2.5
EDTA	20	99
α,α'-Dipyridyl	1.0	100

Chemical	Concn.(mM)	Residual activity(%)
NaF	1.0	105
PCMB	0.33	3.3
MIA	1.0	106
IAA	1.0	103
NaN_3	10	106
o-Phenanthroline	1.0	108
Hydroxylamine	1.0	105
NEM	10	0.3
Triton X-100	0.5%	94
Brij 35	0.5%	103
Tween 20	0.5%	100
Span 20	0.5%	106
Na-cholate	0.5%	103
SDS	0.25%	102
DAC	0.5%	1.7

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

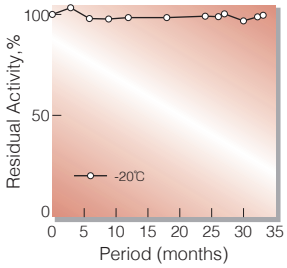


Fig.1. Stability (Powder form)

〔kept under dry conditions〕

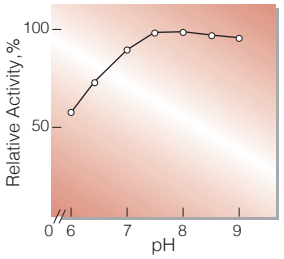


Fig.4. pH-Activity

〔37℃ ,10min-reaction in 50mM K-phoshate buffer〕

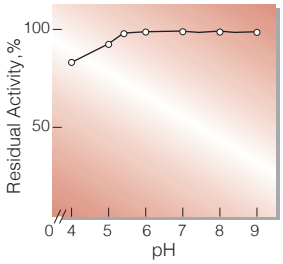


Fig.6.pH-Stability

〔25℃,16hr-treatment with 50mM buffer solution: pH4.0-5.5, Acetate pH6.0-9.0, K-phosphate〕

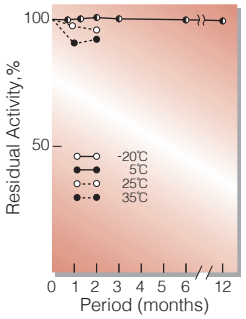


Fig.2. Stability (Powder form)

〔kept under dry conditions〕

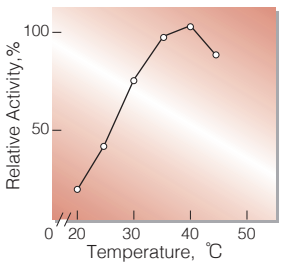


Fig.5.Temperature activity

〔10min-reaction in 50mM K-phosphate buffer, pH7.5〕

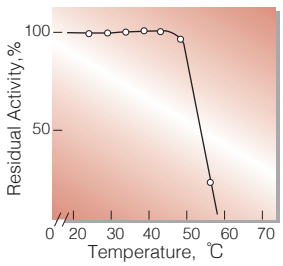


Fig.7. Thermal stability

〔30min-treament with 50mM K-phosphate buffer, pH7.5〕

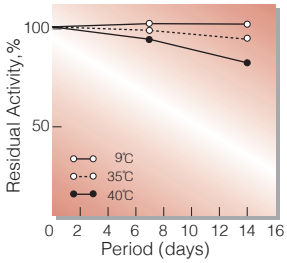


Fig.3. Stability (Liquid form)

〔in 50 mM K-phosphate buffer, pH7.5〕

活性測定法（Japanese）

1.原理

Creatine＋H_2O $\xrightarrow{\text{creatine amidinohydrolase}}$ Sarcosine＋Urea

Urea＋2$(CH_3)_2N$-  -CHO ⟶〔$(CH_3)_2N$-  -C＝N〕$_2CO$＋2H_2O

生成した尿素のp-ジメチルアミノベンズアルデヒド(DAB)との縮合(Ehrich反応)生成物(黄色色素)を比色定量する。

2.定義

下記条件下で1分間に1マイクロモルの黄色色素を生成する酵素量を1単位(U)とする。

3.試薬

A. 0.1Mクレアチン溶液〔1.49gのクレアチン(ナカライテスク製)を50mMリン酸緩衝液 pH7.5に溶解し,100mℓとする〕(用時調製)

B. DAB溶液(2.0gのp-ジメチルアミノベンズアルデヒドを100mℓのジメチルスルホキシドに溶解させた後,濃塩酸15mℓを加える)

酵素溶液：酵素標品を予め氷冷した50mMリン酸緩衝液,pH7.5で溶解し,分析直前に同緩衝液で2.0～3.0U/mℓに希釈する。

4.手順

①試験管に基質溶液(A)1.0mℓを採り,37℃で約5分間予備加温する。
②酵素溶液0.1mℓを加え,反応を開始する。
③37℃で正確に10分間反応させた後,DAB溶液(B)2.0mℓを加えて反応を停止させる。
④25℃で20分間放置後,435nmにおける吸光度を測定する (ODtest)。
⑤盲検は基質溶液(A)1.0mℓを37℃で10分間放置後,DAB溶液(B)2.0mℓを加えて混和し,次いで酵素溶液0.1mℓを加えて調製する。以下同様に25℃で20分間放置後吸光度を測定する(ODblank)。

5.計算式

$$U/m\ell = \frac{\Delta OD(OD\ test - OD\ blank) \times 3.1(m\ell) \times 希釈倍率}{0.321 \times 1.0 \times 10(分) \times 0.1(m\ell)}$$

$$= \Delta OD \times 9.65 \times 希釈倍率$$

U/mg ＝U/mℓ×1/C

0.321 ： 黄色色素のミリモル分子吸光係数 (㎠/micromole)

1.0 ： 光路長(cm)

C ： 溶解時の酵素濃度(c mg/mℓ)