Creatininase (CAH) from Microorganism

C1492996

Storage temperature: -20℃.

Introduction:

Creatinine Amidohydrolase catalyzes the hydrolytic reaction converting creatinine to creatine. The enzyme is purified from a microorganism. The molecular size of the enzyme is approximately 175,000. The enzyme is useful for the enzy-matic assay of creatinine when coupled with other related enzymes.

Reaction formula: Creatinine + H_2O → Creatine

PREPARATION and SPECIFICATION

Appearance	White amorphous powder, lyophilized
Activity	≥450 U/mg powder
Contaminants	NADH oxidase ≤5.0×10^{-2}%; Catalase ≤2.0%
Stabilizers	Sucrose, BSA

PROPERTIES

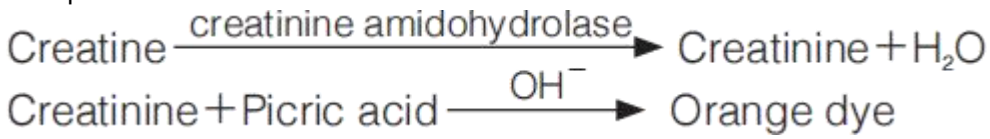
Stability	Stable at -20℃ for at least one year	(Fig.1,2)
Molecular weight	approx. 175,000	
Isoelectric point	4.7	
Michaellis constants	3.2×10^{-3} M (Creatinine), 5.7×10^{-3} M (Creatine)	
Structure	6 subunits per enzyme molecule (One zinc is bound to each subunit)	
Inhibitors	Ag^{+}, Hg^{++}, N-bromosuccinimide, EDTA	
Optimum pH	6.5 - 7.5	(Fig.5)
Optimum temperature	70℃	(Fig.6)
pH Stability	pH 7.5 - 9.0 (5℃, 16hr)	(Fig.7)
Thermal stability	below 70℃ (pH 7.5, 30min)	(Fig.8)
Effect of various metals	-	(Table 1)

APPLICATIONS

This enzyme is useful for enzymatic determination of creatinine when coupled with creatine amidinohydrolase (CRH-211, CRH-221, CRH-229) and sarcosine oxidase (SAO-351) in clinical analysis.

ASSAY

Principle:



The appearance of creatinine-picrate (orange dye based on Jaffe's reaction) is measured at 520nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of orange 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. NaOH solution: 0.5N

C. Picric acid solution: 1.0% (W/V)

D. 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℃, immediately transfer an aliquot (0.1ml) (1/11 volume) of the reaction solution to 2.0ml of NaOH solution (B).
4. Add 1.0ml of picric acid solution (C) and incubate at 25℃ for 20 minutes.
5. Measure the optical density at 520nm against water (OD test).

At the same time, prepare the blank by transferring an aliquot (0.1ml) of the reaction solution into NaOH solution (2.0ml) just after the addition of the enzyme solution into ice-cold substrate solution, and carry out the same procedure as the test (procedure 4 and 5) (OD blank).

*Dissolve the enzyme preparation in ice-cold 50mM phosphate buffer, pH 7.5 and dilute to 1.8−2.4U/ml with the same buffer, immediately before assay.

Concentration in assay mixture

Phosphate buffer	45mM
Creatine	90mM

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt \times 11 \times df}{4.65 \times 1.0 \times t \times Vs} = \Delta OD \times 7.33 \times df$$

$$\text{Weight activity (U/mg)} = (U/ml) \times 1/C$$

Vt: Total volume (3.1ml)

Vs: Sample volume (0.1ml)

4.65: Millimolar extinction coefficient of creatinine-picrate (orange dye) (cm^2/micromole)

11: Volume of reaction solution (1.1ml)/Sampling volume (0.1ml)

1.0: Light path length (cm)

t: Reaction time (10 minutes)

df: Dilution factor of enzyme solution

C: Enzyme concentration in dissolution (mg/ml)

Table 1. Effect of Various Metals on Creatinine amidohydrolase

[The enzyme dissolved in 50mM K-phosphate buffer, pH 7.5 was incubated with each metal (0.2mM) for 30 minutes at 25℃.]

Metal	Residual activity(%)	Metal	Residual activity(%)
None	100	$MgCl_2$	82.8
$FeSO_4$	91.0	$NiCl_2$	82.8
$FeCl_3$	93.0	$CoSO_4$	78.5
$HgCl_2$	1.4	$BaCl_2$	71.2
$Zn(OAc)_2$	79.9	$Pb(OAc)_2$	88.0
$Cu(OAc)_2$	22.7	$AgNO_3$	0
$Ca(OAc)_2$	96.8	$CdCl_2$	80.0
$MnCl_2$	94.0		

Ac, CH_3CO.

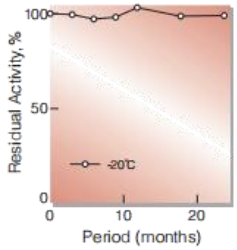


Fig.1. Stability (CNH-211) (Powder form)
(kept under dry conditions)

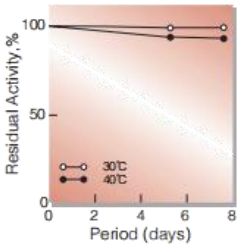


Fig.4. Stability (Liquid form)
(in 50 mM Tris-HCl buffer solution pH7.5)

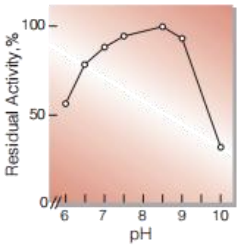


Fig.7. pH-Stability
(25℃,16hr-treatment with 50mM buffer solution: pH6.0-8.0, phosphate; pH8.5-9.0, carbonate)

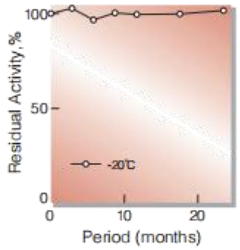


Fig.2. Stability (CNH-311) (Powder form)
(kept under dry conditions)

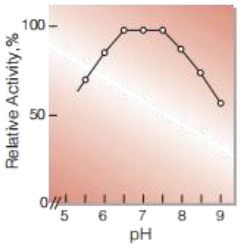


Fig.5. pH-Activity
(37℃, 10min-reaction in 50mM buffer solution: pH5.5, acetate;pH6.0-8.0,phosphate; pH8.5-9.0,carbonate)

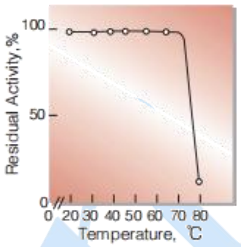


Fig.8. Thermal stability
(30min-treatment with 50mM phosphate buffer, pH7.4)

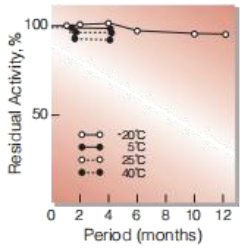


Fig.3. Stability (Powder form)
(kept under dry conditions)

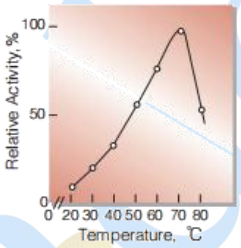


Fig.6. Temperature activity
(10min-reaction in 50mM phosphate buffer, pH7.4)

aladdin