Urease from Canavalia ensiformis (Jack bean)

U754676

Storage temperature: -20℃.

Introduction

Urease from Canavalia ensiformis (Jack bean) is derived from jack bean and Catalyzes hydrolysis of urea to carbon dioxide and ammonia. Urease, Jack bean is useful in the determination of urea in body fluids.

PREPARATION and SPECIFICATION

Appearance: White amorphous powder, lyophilized

Activity: Grade Ⅱ (-201) 100U/mg-solid or more

Contaminants:

Asparaginase ≤2.0×10^{-3} %

Arginase: ≤2.0×10^{-3} %

NH_4^+: ≤5.0×10^{-4} μg / U

Stabilizers: EDTA, glutathione, succinate, BSA

Properties

Stability	Stable at -20°C for at least one year	(Fig. 1)
Molecular Weight	Approximately 480,000	
Isoelectric Point	5.0 - 5.1	
Michaelis Constant	1.05×10^{-2} M (Urea)	
Structure	8 active sites with SH-groups per enzyme molecule	
Inhibitors	Heavy metal ions (Ag^+, Hg^{2+}, etc.)	
Optimum pH	6.0	(Fig. 3)
Optimum Temperature	60°C	(Fig. 4)
pH Stability	pH 5.5 - 8.5 (30°C, 17 hours)	(Fig. 5)
Thermal Stability	Stable below 50°C (pH 8.0, 60 minutes)	(Fig. 6)
Effect of Various Chemicals		(Table 1)

APPLICATIONS

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 elimination of NADPH is measured at 340 nm by spectrophotometry.

Unit definition

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

Method

Reagents:

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

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

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

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

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

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

F. GIDH (Glutamate Dehydrogenase) Solution: ca.1,000 U/ml (Tris-HCl buffer solution, free from ammonia).

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

Procedure:

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

2.40 mL Working solution (E)

0.05 mL GIDH solution (F)

0.35 mL H_2O

0.10 mL Enzyme solution*

Concentration in Assay Mixture

Tris-HCl Buffer	38 mM
Urea	200 mM
α-Ketoglutarate	0.83 mM
NADPH	0.30 mM
EDTA	0.67 mM
GIDH	ca.17 U/ml

2. Add 0.10 ml of Urea Solution (A) and mix by gentle inversion.
3. Record the decrease in optical density at 340 nm against water for 3 to 4 minutes using a spectrophotometer thermostated at 37°C. Calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

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

Calculation

Activity can be calculated using the following formulas:

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

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

Vt: Total volume (3.0 ml).

Vs: Sample volume (0.10 ml).

6.22: Millimolar extinction coefficient of NADPH at 340 nm (cm^2/micromole).

2: Factor based on the fact that the hydrolysis of 1 mole of urea is equivalent to the oxidation of 2 moles of NADPH.

1.0: Light path length (cm).

df: Dilution factor.

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

Table 1: Effect of Various Chemicals on Urease

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

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
$Na_2B_4O_7$	10	104	$HgCl_2$	0.1	8
$Na_2S_2O_4$	10	108			

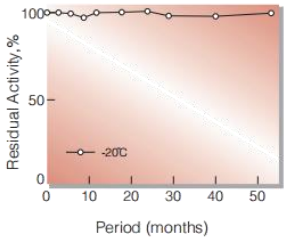

Fig.1. Stability (Powder form)

〔kept under dry conditions〕

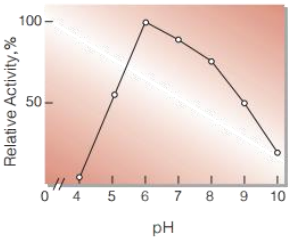

Fig.3. pH-Activity

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

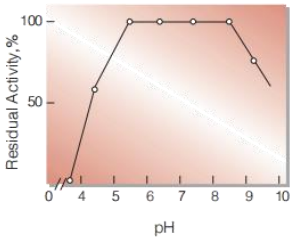

Fig.5. pH-Stability

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

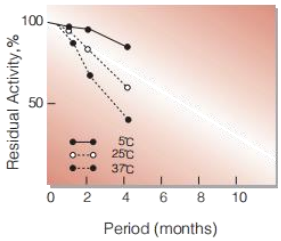

Fig.2. Stability (Powder form)

〔kept under dry conditions〕

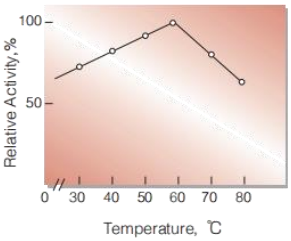

Fig.4. Temperature activity

〔in 20mM phosphate buffer,pH7.0〕

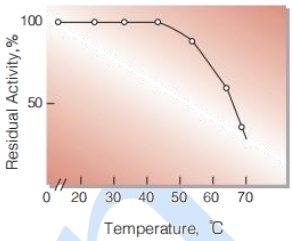

Fig.6. Thermal stability

〔60min-treatment with 20mM phosphate buffer, pH8.0.〕