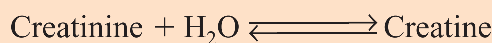
Creatininase (C1-E)

from recombinant *E. coli*

Creatinine amidohydrolase, EC 3.5.2.10

Creatinine + H_2O  Creatine

SPECIFICATION

Appearance	white lyophilizate
Activity	≧500 U/mg lyophilizate
Contaminant	catalase ≦1.0%
Stabilizer	sucrose
Storage	at −20°C

PROPERTIES

Molecular weight	*ca*. 170 kDa (gel filtration)	
Structure	6 subunits of 28 kDa (SDS-PAGE)	
Isoelectric point	4.8	
Michaelis constants	3.4×10^{-2} M (creatinine)	
	4.3×10^{-2} M (creatine)	
pH Optimum	6.5–7.0	(Fig. 1)
pH Stability	7.0–11.0	(Fig. 2)
Optimum temperature	60–65°C	(Fig. 3)
Thermal stability	below 60°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least two weeks	(Fig. 5)
Stability (powder form)	stable at 30°C for at least one month	(Fig. 6)
Inhibitor	Hg^{2+}	
Activators	Mg^{2+}, Mn^{2+}	

ASSAY PROCEDURE

Principle

Creatinine + H_2O $\xrightarrow{\text{creatininase}}$ Creatine

The appearance of creatine is measured spectrophotometrically at 525 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of creatine per min at 37°C and pH 6.8 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 0.3 M; pH 6.5: mix 0.3 M KH_2PO_4 solution and 0.3 M K_2HPO_4 solution to make a pH 6.5 solution.
B. Creatinine solution, 0.1 M: 1.13 g of creatinine/100 ml of distilled water.
C. Sodium carbonate solution, 4%: 4.0 g of Na_2CO_3 (anhydrous)/100 ml of distilled water.
D. α-Naphthol solution, 2%: 2.0 g of α-naphthol/100 ml of ethanol (99.5%).
E. Alkaline solution, 1.2% NaOH, 3.2% Na_2CO_3: dissolve 1.2 g of NaOH and 3.2 g of Na_2CO_3 (anhydrous) in 80 ml of distilled water and dilute with distilled water to 100 ml.
F. Diacetyl solution, 0.05%: 0.05 ml of diacetyl/100 ml of distilled water.
G. Enzyme dilution buffer: dissolve 61 mg of Tris(hydroxymethyl)aminomethane in 80 ml of distilled water, adjust to pH 8.0 with 1 N HCl and dilute with distilled water to 100 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 2–4 U/ml with ice-cold enzyme dilution buffer (Reagent G) immediately before measurement.

Procedure

1. Pipette the following reagents into a test tube.

0.1 ml	Potassium phosphate buffer	(Reagent A)
0.8 ml	Creatinine solution	(Reagent B)

2. Equilibrate at 37°C for about 5 min.
3. Add 0.1 ml of sample and incubate for 10 min at 37°C.
4. Add 2.0 ml of sodium carbonate solution (Reagent C) to stop the reaction and cool in ice water.
5. Pipette successively the following reagents into a test tube.

0.1 ml	The terminated solution of step 4	
0.9 ml	Distilled water	
0.5 ml	α-Naphthol solution	(Reagent D)
0.5 ml	Alkaline solution	(Reagent E)
0.5 ml	Diacetyl solution	(Reagent F)

6. Allow to stand for about 1 h at 25°C and dilute by adding 2.5 ml of distilled water.
7. Read the absorbance at 525 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by reversing the sequence of addition of sample and sodium carbonate solution (Reagent C) (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_S - A_0) \times 1.0(\text{ml}) \times df}{0.0704 \times 0.1(\text{ml}) \times 10(\text{min})} = \Delta A \times 14.2 \times df$$

Weight activity (U/mg) = (U/ml) × 1/C

0.0704 : Millimolar extinction coefficient of creatine under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of creatininase preparation in sample (mg/ml)

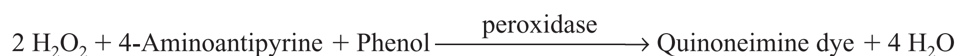
APPLICATIONS

The enzyme is useful for the determination of creatinine in clinical analysis.

Creatinine + H_2O —creatininase→ Creatine

Creatine + H_2O —creatinase→ Sarcosine + Urea

Sarcosine + H_2O + O_2 —sarcosine oxidase→ Glycine + Formaldehyde + H_2O_2

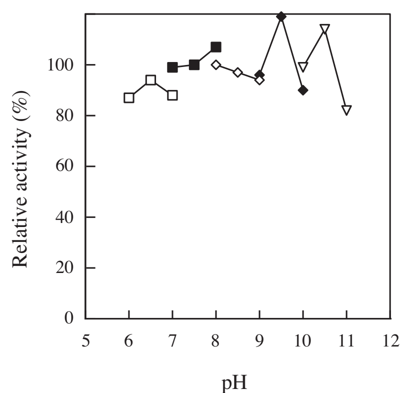

2 H_2O_2 + 4-Aminoantipyrine + Phenol —peroxidase→ Quinoneimine dye + 4 H_2O

REFERENCE

Suzuki, M. and Yoshida, M., *Clin. Chim. Acta*, **143**, 147–155 (1984).

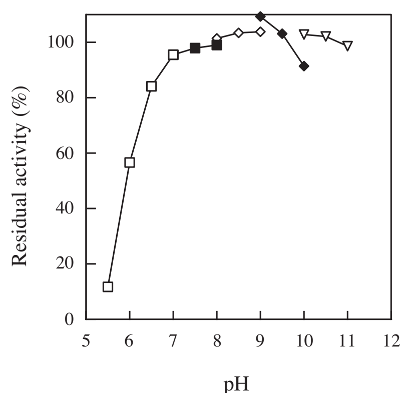
EXPERIMENTAL DATA

Fig. 1 pH Optimum



□: 30 mM MES–NaOH buffer
■: 30 mM HEPES–NaOH buffer
◇: 30 mM TAPS–NaOH buffer
◆: 30 mM CHES–NaOH buffer
▽: 30 mM CAPS–NaOH buffer

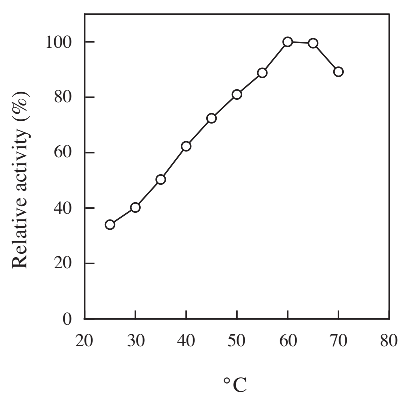
Fig. 2 pH Stability



Treatment: 5°C, 24 h

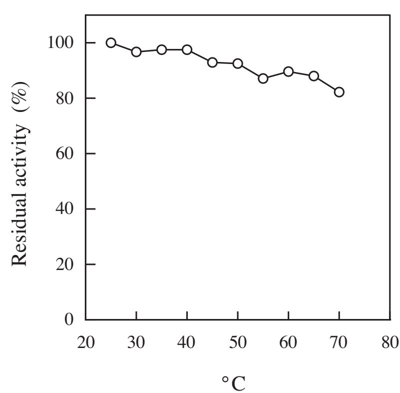
□: 30 mM MES–NaOH buffer
■: 30 mM HEPES–NaOH buffer
◇: 30 mM TAPS–NaOH buffer
◆: 30 mM CHES–NaOH buffer
▽: 30 mM CAPS–NaOH buffer

Fig. 3 Optimum temperature



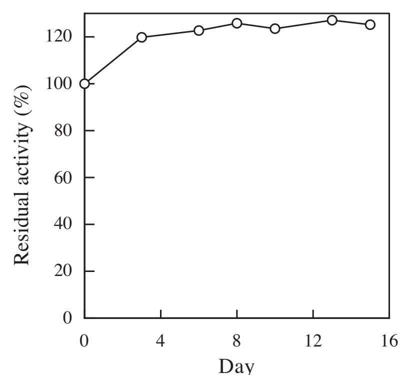
Buffer: 30 mM phosphate buffer, pH 6.5

Fig. 4 Thermal stability



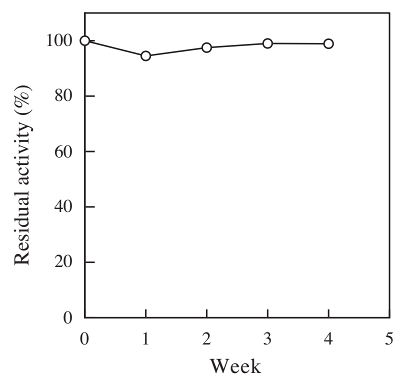
Treatment: 50 mM MES–NaOH buffer, pH 6.5, 10 min

Fig. 5 Stability (liquid form) at 37°C



[Kept in 5 mM Tris-HCl buffer, pH 8.0, containing 6% sucrose]

Fig. 6 Stability (powder form) at 30°C



(Kept under dry conditions)