Creatinase (C2-AT)

from recombinant *E. coli*

Creatine amidinohydrolase, EC 3.5.3.3

$$\text{Creatine} + H_2O \longrightarrow \text{Sarcosine} + \text{Urea}$$

SPECIFICATION

Appearance	white lyophilizate	
Activity	≧9 U/mg lyophilizate	
Contaminant	catalase	≦0.5%
Stabilizer	sucrose	
Storage	at −20°C	

PROPERTIES

Molecular weight	*ca*. 80 kDa (gel filtration)	
Structure	2 subunits of 48 kDa (SDS-PAGE)	
Michaelis constant	8.6×10^{-3} M (creatine)	
pH Optimum	7.0–9.0	(Fig. 1)
pH Stability	4.0–11.0	(Fig. 2)
Optimum temperature	45°C	(Fig. 3)
Thermal stability	below 53°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+}	

ASSAY PROCEDURE

Principle

$$\text{Creatine} + H_2O \xrightarrow{\text{creatinase}} \text{Sarcosine} + \text{Urea}$$

The appearance of urea is measured spectrophotometrically at 435 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of urea per min at 37°C and pH 7.7 under the conditions described below.

Reagents

A. Potassium phosphate buffer, 0.3 M; pH 7.7: mix 0.3 M KH_2PO_4 solution and 0.3 M K_2HPO_4 solution to make a pH 7.7 solution.
B. Creatine solution, 0.1 M: 1.49 g of creatine monohydrate/100 ml of distilled water.
C. *p*-Dimethylaminobenzaldehyde (DMAB) solution, 0.87%: dissolve 2.0 g of DMAB in 100 ml of ethanol (99.5%) and add 15 ml of conc. HCl and 115 ml of distilled water.
D. Enzyme dilution buffer: mix 10 mM KH_2PO_4 solution and 10 mM K_2HPO_4 solution to make a pH 8.0 solution and add 2-mercaptoethanol (0.16 ml/liter of the buffer).

Sample: dissolve the lyophilized enzyme to a volume activity of 1.4–2.8 U/ml with ice-cold enzyme dilution buffer (Reagent D) immediately before measurement.

Procedure

1. Pipette the following reagents into a test tube.
   0.1 ml Potassium phosphate buffer (Reagent A)
   0.8 ml Creatine 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 DMAB solution (Reagent C) and allow to stand for about 30 min at 25°C.
5. Read the absorbance at 435 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by reversing the sequence of addition of sample and DMAB solution (Reagent C) (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_S - A_0) \times 1.0(\text{ml}) \times df}{0.0543 \times 0.1(\text{ml}) \times 10(\text{min})} = \Delta A \times 18.4 \times df$$

Weight activity (U/mg) = (U/ml) × 1/*C*

0.0543 : Millimolar extinction coefficient of urea under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of creatinase preparation in sample (mg/ml)

APPLICATIONS

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

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

$$\text{Creatine} + H_2O \xrightarrow{\text{creatinase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O + O_2 \xrightarrow{\text{sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H_2O_2$$

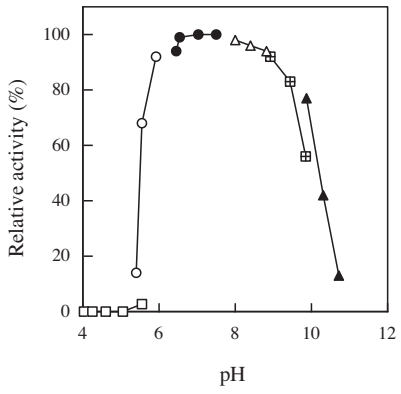
$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\ H_2O$$

REFERENCES

Suzuki, M., *Medical Technology*, **7**, 945–950 (1979).
Suzuki, M. and Yoshida, M., *Clin. Chim. Acta*, **140**, 289–294 (1984).
Suzuki, M. and Yoshida, M., *Clin. Chim. Acta*, **143**, 147–155 (1984).

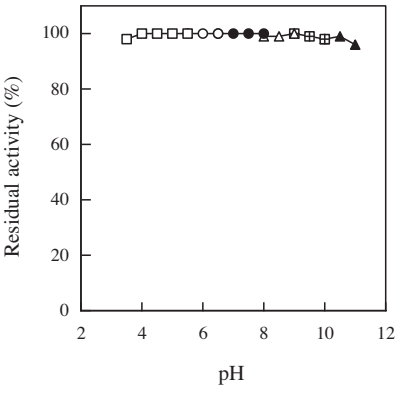
EXPERIMENTAL DATA

Fig. 1 pH Optimum



□: 50 mM acetate buffer
○: 50 mM MES–NaOH buffer
●: 50 mM phosphate buffer
△: 50 mM Tris–HCl buffer
⊞: 50 mM HEPES–NaOH buffer
▲: 50 mM CAPS–NaOH buffer

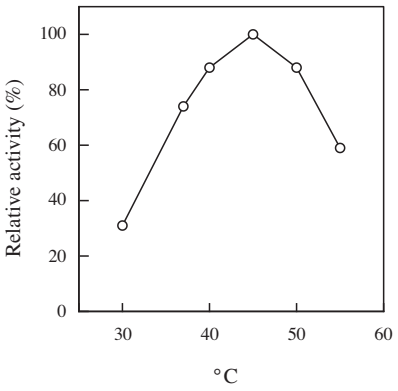
Fig. 2 pH Stability



Treatment: 25°C, 17 h

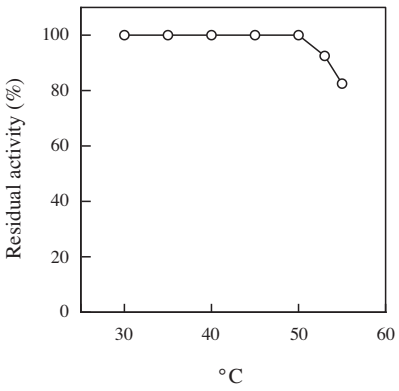
□: 50 mM acetate buffer
○: 50 mM MES–NaOH buffer
●: 50 mM phosphate buffer
△: 50 mM Tris–HCl buffer
⊞: 50 mM HEPES–NaOH buffer
▲: 50 mM CAPS–NaOH buffer

Fig. 3 Optimum temperature



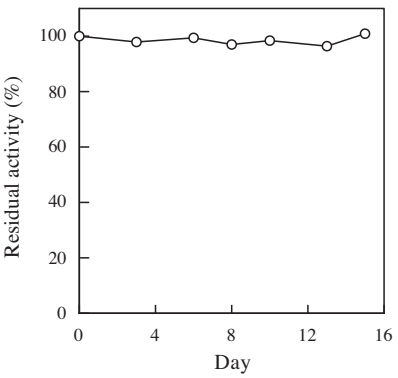
Buffer: 30 mM phosphate buffer, pH 7.7

Fig. 4 Thermal stability



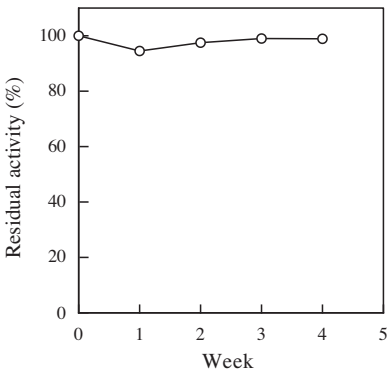
Treatment: 50 mM Tris–HCl buffer, pH 7.5, 30 min

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



Kept in 10 mM potassium phosphate buffer, pH 8.0, containing 5% sucrose

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



(Kept under dry conditions)