Creatinase (C2-AE)

from recombinant *E. coli*

Creatine amidinohydrolase, EC 3.5.3.3

Creatine + H₂O → Sarcosine + 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 46 kDa (SDS-PAGE)
- **Michaelis constant**: 1.3×10⁻² M (creatine)
- **pH Optimum**: 7.0–9.0 (Fig. 1)
- **pH Stability**: 5.0–11.0 (Fig. 2)
- **Optimum temperature**: 40°C (Fig. 3)
- **Thermal stability**: below 45°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**: Hg²⁺
ASSAY PROCEDURE

Principle

Creatine + H₂O ⇌ Sarcosine + 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 KH₂PO₄ solution and 0.3 M K₂HPO₄ solution to make a pH 7.7 solution.
B. Creatine solution, 0.1 M: dissolve 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 KH₂PO₄ solution and 10 mM K₂HPO₄ solution to make a pH 8.0 solution and add 2-mercaptoethanol (0.16 ml/l 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 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) (A₅₃₅).
   The blank solution is prepared by reversing the sequence of addition of sample and DMAB solution (Reagent C) (A₅₃₅₀).
Calculation

Activity can be calculated by using the following formula:

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

Weight activity (U/mg) = (U/ml) \times 1/C

0.0543 : Millimolar extinction coefficient of urea under the assay conditions (cm²/μmol)

\(df\) : 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.

Creatinine + H₂O \xrightarrow{\text{creatinase}} \text{Creatine}

Creatine + H₂O \xrightarrow{\text{creatinase}} \text{Sarcosine} + \text{Urea}

Sarcosine + H₂O + O₂ \xrightarrow{\text{sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + H₂O₂

2 H₂O₂ + 4-Aminoantipyrine + Phenol \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4 H₂O

REFERENCES

Experimental Data

Fig. 1  pH Optimum

- Relative activity (%)
- pH
- 50 mM acetate buffer
- 50 mM phosphate buffer
- 50 mM Tris–HCl buffer

Fig. 2  pH Stability

- Residual activity (%)
- pH
- 50 mM acetate buffer
- 50 mM phosphate buffer
- 50 mM Tris–HCl buffer
- 50 mM glycine–NaOH buffer
- 50 mM CAPS–NaOH buffer

Fig. 3  Optimum temperature

- Relative activity (%)
- °C
- Buffer: 50 mM phosphate buffer, pH 7.5

Fig. 4  Thermal stability

- Residual activity (%)
- °C
- Treatment: 50 mM Tris–HCl buffer, pH 7.5, 30 min

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

- Residual activity (%)
- Day
- Kept in 10 mM potassium phosphate buffer, pH 8.0, containing 5% sucrose

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

- Residual activity (%)
- Week
- (Kept under dry conditions)