Creatine amidinohydrolase (CR)

rp216175

Preparation and specification:

Appearance	White amorphous powder,lyophilized
Protein purity	⩾90% (from SDS-PAGE)
Activity	⩾5.8 U/mg solid
Catalase	⩽0.1%
Creatine amidinohydrolase	⩽0.01%
Sarcosine oxidase	⩽0.01%
NADH/NADPH oxidase	⩽0.01%

Stability and Storage

Store at -20℃ long term (12 months). Upon delivery aliquot. Avoid freeze/thaw cycle.

Properties

EC number	3.5.3.3(Recombinant from microorganism)	
Molecular weight	48 kDa (SDS-PAGE)	
Isoelectric point	4.6	
Michaelis constants	8.81×10^{-3} M (Creatine)	
Inhibitors	Ag^{+}, Cu^{2+}, Hg^{2+}	
Optimum pH	8.0	Fig. 1
Optimum temperature	45℃	Fig. 2
pH stability	pH 4.5-10.0 (25℃, 16h)	Fig. 3
Thermal stability	Below 50℃(pH 7.5, 30 min)	Fig. 4
Stabilizers	EDTA, sugar	

Applications

This enzyme is useful for enzymatic determination of creatinine when coupled with creatine amidohydrolase and sarcosine oxidase

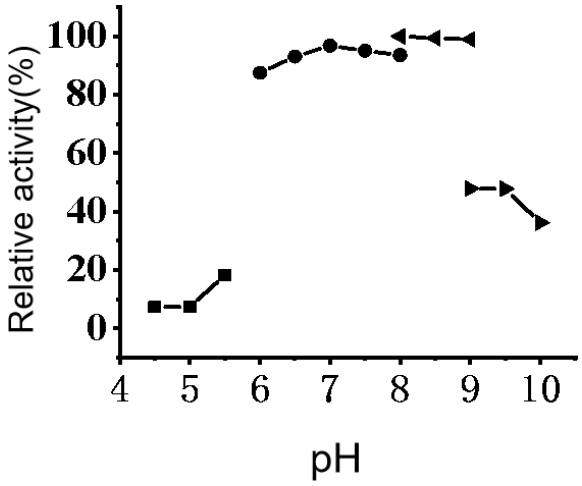


Fig. 1 Optimum pH

50 mM Buffer solution: pH 4.5-5.5, acetate buffer; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

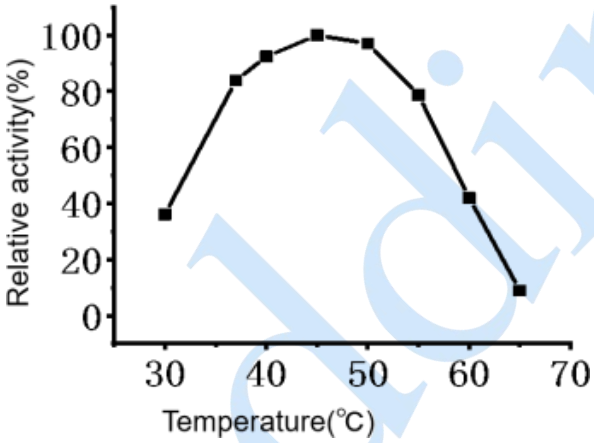


Fig. 2 Optimum temperature

Reaction in 50 mM Na-phosphate buffer pH 7.5. Enzyme concentration: 1 mg/mL

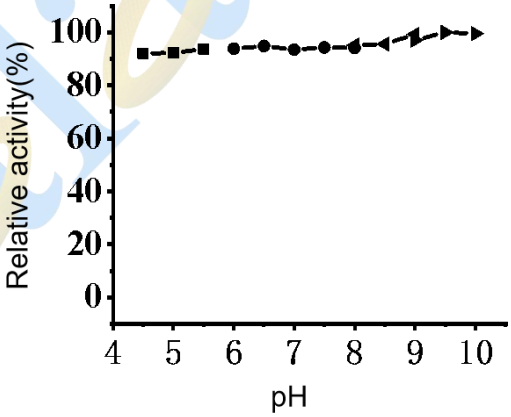


Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, acetate buffer; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1

mg/mL

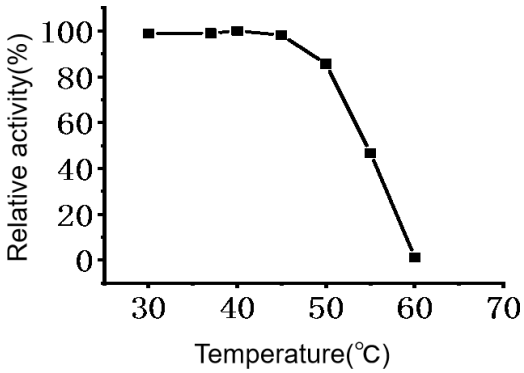
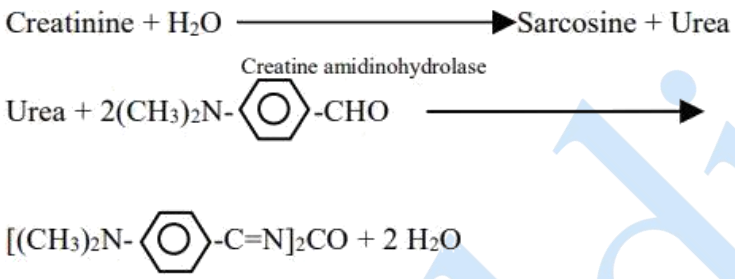


Fig. 4 Thermal stability

30 min treatment with 50 mM Na-phosphate buffer, pH 7.5.Enzyme concentration: 1 mg/mL

Assay principle



The appearance of yellow dye formed by condensation of urea and p-dimethylaminobenzaldehyde (PDAB) (Ehrlich reaction) is measured at 435nm by spectrophotometry.

Unit definition

One unit (U) is the amount of enzyme that produces 1 μmol of yellow dye per min under the conditions described below.

Reagents preparation

Reagent I: 0.1M Creatine solution.

Reagent II: DAB solution: dissolve 2.0 g of DAB in 100 mL of dimethyl sulfoxide (DMSO), then add 15 mL of concentrated hydrochloric acid.

Enzyme diluent: 50 mM NaH_2PO_4-Na_2HPO_4 buffer, pH 7.5.

Enzyme solution: dilute the enzyme to 2.0-3.0 U/ml with enzyme diluent.

Procedure

1.Pipette 1.0 ml of reagent I into a test tube and equilibrate at 37 ℃ for about 5 minutes.

2.Add 0.1 ml of the enzyme solution and mix.

3.After 10 minutes at 37 ℃, add 2.0 ml of reagent II to stop the reaction.

4.Incubate at 25 ℃ for 20 minutes. Measure the optical density As at 435 nm.

At the same time, prepare the blank by first mixing the substrate solution with 2.0 ml of DAB solution after 0 min-incubation at 37 ℃, followed by the addition of the enzyme solution, and carry out the same procedure as test (procedure 4 and 5) (A_b).

$\Delta A = A_S - A_b$

Calculation

$$\text{Volume activity (U/mL)} = \frac{\Delta A \times 3.1 \times df}{0.321 \times 0.1 \times 1.0 \times 10} = \Delta A \times 9.66 \times df$$

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

3.1: Total volume (mL)
0.1: Enzyme volume (mL)
1.0: Light path length (cm)
10: Reaction time (minutes)
df: Dilution factor
C: Enzyme concentration (mg/mL)
0.321: Millimolar extinction coefficient of yellow dye under 435nm ($cm^2/\mu mol$)

References

1.D. Tsuru; Nucleic Acid and Amino Acids, 35, 31 (1977).
2.T.Yoshimoto, I.Oka and D.Tsuru; Arch. Biochem. Biophys., 177, 508 (1976).
3.T. Yoshimoto, I. Oka and D. Tsuru; J. Biochem., 79, 1381 (1976).
4.D. Tsuru; Rinsho Kensa, 22, 1331 (1978).