Creatinine amidohydrolase (CAH)

rp216173

Preparation and specification:

Appearance	White to slightly yellowish amorphous powder,lyophilized
Purity(SDS-PAGE)	⩾90.0%
Enzyme powder specific activity	⩾400.0 U/mg
Catalase	⩽0.1%
ATPase	⩽0.001%
Proteases	⩽0.001%
Creatine amidinohydrolase	⩽0.001%

Properties

EC number	3.5.2.10 (Recombinant from microorganism)	
Molecular weight	29 kDa (SDS-PAGE)	
Isoelectric point	5.3	
Michaelis Constants	5.0×10^{-2} M (Creatinine), 8.0×10^{-2} M (Creatine)	
Inhibitors	Hg^{2+}, Cu^{2+}, Fe^{3+}	
Optimum pH	7.0-8.0	Fig. 1
Optimum temperature	65℃	Fig. 2
pH stability	pH 5.5-10.0 (25 ℃, 16 h)	Fig. 3
Thermal stability	Below 65 ℃(pH 8.0, 30 min)	Fig. 4
Storage stability	At least one year at -25 ~-15 ℃	Fig. 5
Stabilizers	BSA, sugar	

Applications

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

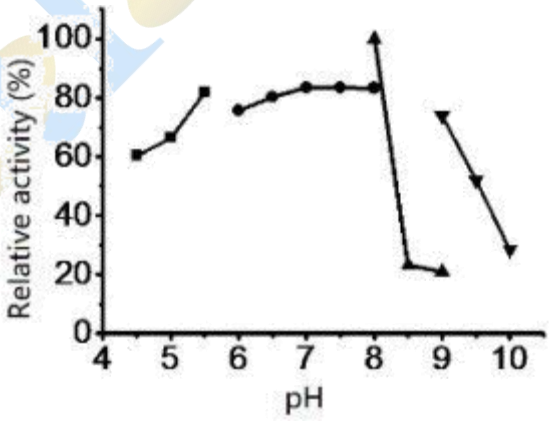


Fig. 1 Optimum pH

300 mM buffer solution: pH 4.5-6.0, acetate buffer; pH 6.5-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

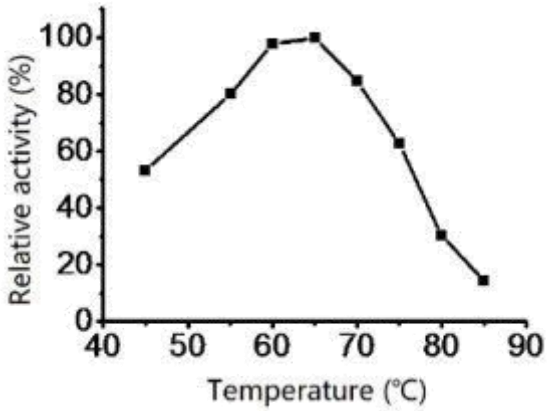


Fig. 2 Optimum temperature

Reaction in 50mM Tris-HCl buffer pH 8.0. Enzyme concentration: 1 mg/mL

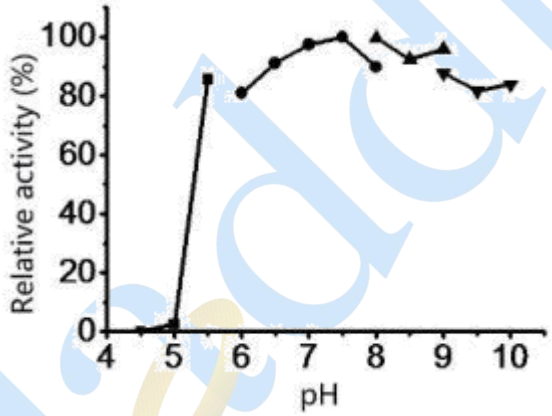


Fig. 3 pH Stability

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

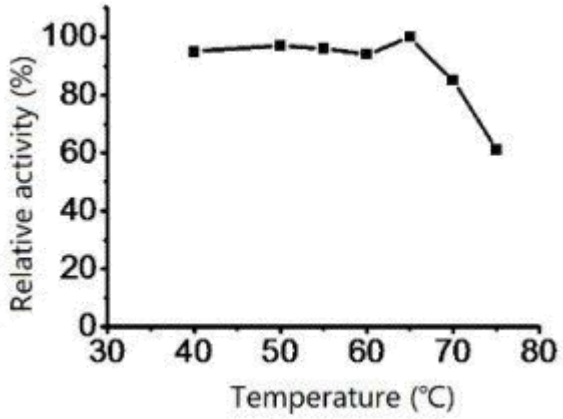


Fig. 4 Thermal stability

30 min-treatment with 100mMTris-HCl buffer pH8.0. Enzyme concentration: 1 mg/mL

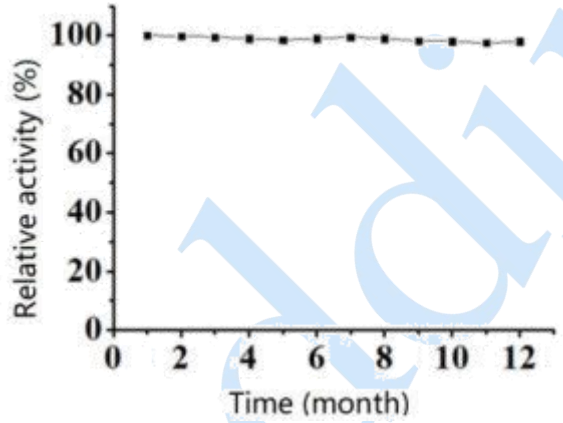
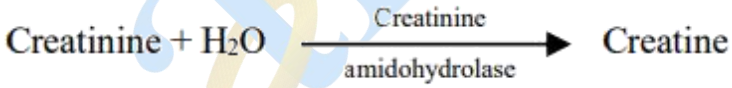


Fig.5 Storage stability (-25 ~ -15℃)

Assay principle



The appearance of creatine is measuredat 525nm by spectrophotometry.

Unit definition

One unit (U) is defined as the amount of enzymewhich produces 1 μ mol of creatineperminunder the conditions described below

Reagents preparation

Reagent I: Potassium phosphate buffer, 0.3 M,pH 6.5.

Reagent II: Creatinine solution, 0.1 M.

Reagent III: Sodium carbonate solution, 4%.

Reagent IV: a-Naphthol solution, 2%.

Reagent V: Alkaline solution, 1.2% NaOH, 3.2% Na_2CO_3.

Reagent VI: Diacetyl solution, 0.05%.

Enzyme diluent: 5 mM Tris-HCl, pH 8.0.

Sample: dilute the enzyme to 2.0−4.0 U/ml with enzyme diluent.

Procedure

1. Pipette 0.1 ml of reagent I and 0.8 ml reagent II into a test tube and equilibrate at 37 ℃ for about 5 minutes.

2. Add 0.1 ml the enzyme solution and mix.

3. After 10 minutes at 37 ℃, add 2.0ml of reagent III to stop the reaction and cool in ice water.

4. Pipette successively the following reagents into a new test tube

0.08 ml	The terminated solution of step 3
0.72 ml	Distilled water
0.4 ml	α-Naphthol solution (Reagent IV)
0.4 ml	Alkaline solution (Reagent V)
0.4 ml	Diacetyl solution (Reagent VI)

5. Allow to stand for about 1 h at 25 °C and dilute by adding 2 ml of distilled water.

6. 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 III) (A_b). $\Delta A = A_S - A_b$

Calculation

$$\text{Volume activity}(U/ml) = \frac{\Delta A \times vt \times df}{0.0704 \times t \times Vs \times 1.0} = \Delta A \times 14.2 \times$$

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

1.0: Total volume (mL)

0.1: Enzyme volume (mL)

1.0: Light path length (cm)

10: Reaction time (10 minutes)

df: Dilution factor

C: Enzyme concentration (mg/mL)

0.0704: Millimolar extinction coefficient of creatine under the assay conditions (cm2 /μmol)

References

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