Creatininase (C1-E)

from recombinant *E. coli*

Creatinine amidohydrolase, EC 3.5.2.10

Creatinine + H₂O $\rightleftharpoons$ Creatine

### SPECIFICATION

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>white lyophilizate</td>
</tr>
<tr>
<td>Activity</td>
<td>$\geq 500$ U/mg lyophilizate</td>
</tr>
<tr>
<td>Contaminant</td>
<td>catalase $\leq 1.0%$</td>
</tr>
<tr>
<td>Stabilizer</td>
<td>sucrose</td>
</tr>
<tr>
<td>Storage</td>
<td>at $-20^\circ$C</td>
</tr>
</tbody>
</table>

### PROPERTIES

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>ca. 170 kDa (gel filtration)</td>
</tr>
<tr>
<td>Structure</td>
<td>6 subunits of 28 kDa (SDS-PAGE)</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.8</td>
</tr>
<tr>
<td>Michaelis constants</td>
<td>$3.4 \times 10^{-2}$ M (creatinine)</td>
</tr>
<tr>
<td></td>
<td>$4.3 \times 10^{-2}$ M (creatine)</td>
</tr>
<tr>
<td>pH Optimum</td>
<td>6.5–7.0 (Fig. 1)</td>
</tr>
<tr>
<td>pH Stability</td>
<td>7.0–11.0 (Fig. 2)</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>60–65°C (Fig. 3)</td>
</tr>
<tr>
<td>Thermal stability</td>
<td>below 60°C (Fig. 4)</td>
</tr>
<tr>
<td>Stability (liquid form)</td>
<td>stable at 37°C for at least two weeks (Fig. 5)</td>
</tr>
<tr>
<td>Stability (powder form)</td>
<td>stable at 30°C for at least one month (Fig. 6)</td>
</tr>
<tr>
<td>Inhibitor</td>
<td>Hg²⁺</td>
</tr>
<tr>
<td>Activators</td>
<td>Mg²⁺, Mn²⁺</td>
</tr>
</tbody>
</table>
ASSAY PROCEDURE

Principle

\[ \text{Creatinine} \xrightarrow{\text{creatininase}} \text{Creatine} \]

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 \(\mu\)mol 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 \(\text{KH}_2\text{PO}_4\) solution and 0.3 M \(\text{K}_2\text{HPO}_4\) 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 \(\text{Na}_2\text{CO}_3\) (anhydrous)/100 ml of distilled water.
D. \(\alpha\)-Naphthol solution, 2%: 2.0 g of \(\alpha\)-naphthol/100 ml of ethanol (99.5%).
E. Alkaline solution, 1.2% \(\text{NaOH}\), 3.2% \(\text{Na}_2\text{CO}_3\): dissolve 1.2 g of \(\text{NaOH}\) and 3.2 g of \(\text{Na}_2\text{CO}_3\) (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 \(\alpha\)-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) \(A_b\).
Calculation

Activity can be calculated by using the following formula:

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

\[
\text{Weight activity (U/mg)} = (U/ml) \times \frac{1}{C}
\]

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

\( df \) : Dilution factor

\( C \) : Content of creatininase preparation in sample (mg/ml)

APPLICATIONS

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

\[
\text{Creatinine} + \text{H}_2\text{O} \xrightarrow{\text{creatinase}} \text{Creatine}
\]

\[
\text{Creatine} + \text{H}_2\text{O} \xrightarrow{\text{creatinase}} \text{Sarcosine} + \text{Urea}
\]

\[
\text{Sarcosine} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{sarcosine oxidase}} \text{Glycine} + \text{Formaldehyde} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\text{H}_2\text{O}
\]

REFERENCE

EXPERIMENTAL DATA

Fig. 1 pH Optimum

Fig. 2 pH Stability

Fig. 3 Optimum temperature

Fig. 4 Thermal stability

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

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