D-Aminoacylase"Amano"

This enzyme specific for the hydrolysis of N-acetyl-D-amino acids and used for the resolution processes for industrial production of optically pure D-amino acids.

Specifications

Acylase activity (DAC method) Not less than 5 MU/g

Origin:  *Escherichia coli*

Catalysis:

$N$-Acetyl-$D,L$-amino acid $\rightarrow$ D-Amino acid + $CH_3COOH + N$-Acetyl-$L$-amino acid

Characteristics

- Molecular weight: 56,000 to 57,000 (SDS-PAGE)
- Isoelectric point: 5.2
- $K_m$ value: 6.67 mM (substrate: methionine)
- Inhibitors: $Cu^{2+}$, $Fe^{3+}$, $Zn^{2+}$
- Optimal pH: 8.0
- Optimal temperature: 45°C
- Stable pH range: 8.5 to 10.0 (30°C, 17h.)
- Stable temperature range: below 50°C (pH 7.0, 15 min.)
- Form: lyophilizate

Handling

The enzyme preparation may irritate the skin and eyes. The dust may cause sensitization when inhaled. Please take precautions to avoid direct contact with the product. In case of contact with the skin or eyes, rinse thoroughly with copious amount of water. Seek medical advice if lung irritation occurs.

Storage

Stored below 10°C in a dry place.

Important

The information and recommendations contained herein are to the best of our knowledge reliable. However, nothing herein is to be construed as a warranty or representation in respect of safety in use, suitability, efficacy, merchantability or otherwise, including freedom from patent infringement. Users shall make their own tests for their particular purpose. We do not accept any liability for any loss, damage or infringement arising from the use of the information and recommendations contained herein.
Performance Characteristics

(1) pH and Activity

![Graph showing pH and activity relationship.]

- Reaction: 37°C
- Citrate-Na$_2$HPO$_4$: pH 3.5 ~ 8.0
- Na$_2$CO$_3$-H$_3$BO$_3$: pH 8.0 ~ 11.0

(2) pH Stability

![Graph showing pH stability.]

- Condition: 30°C for 17 h
- Citrate-Na$_2$HPO$_4$: pH 3.5 ~ 8.0
- Na$_2$CO$_3$-H$_3$BO$_3$: pH 8.0 ~ 11.0

(3) Temperature and Activity

![Graph showing temperature and activity relationship.]

(4) Heat Stability (Solution)

![Graph showing heat stability.]

- Treatment conditions: pH 8.0
- 1h
- 24h

(5) Substrate Specificity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$-acetyl-D, L-methionine</td>
<td>100</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-phenylalanine</td>
<td>77</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-leucine</td>
<td>68</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-tyrosine</td>
<td>20</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-tryptophan</td>
<td>7</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-alanine</td>
<td>4</td>
</tr>
<tr>
<td>$N$-acetyl-D, L-valine</td>
<td>3</td>
</tr>
</tbody>
</table>
Resolution of N-acetyl-D,L-phenylalanine by D-Aminoacylase "Amano"

Reaction

\[
\begin{align*}
\text{N-Ac-D, L-Phe} & \quad \text{d-Phe} & \quad \text{N-Ac-L-Phe} \\
\text{COOH} & \quad \text{COOH} & \quad \text{COOH} \\
\text{NHCOOCH}_3 & \quad \text{NH}_2 & \quad \text{NHCOOCH}_3 \\
20 \text{ mM Tris-HCl (pH 7.5), 30°C} & & \\
\end{align*}
\]

Result

<table>
<thead>
<tr>
<th>Substrate</th>
<th>D-Amino acylase &quot;Amano&quot;</th>
<th>D-Phe (%)</th>
<th>17 h</th>
<th>41 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5M N-Ac-D, L-Phe</td>
<td>540 U/ml (5.2 KU/g of substrate)</td>
<td>47.5%</td>
<td>50.0%</td>
<td></td>
</tr>
<tr>
<td>1M N-Ac-D, L-Phe</td>
<td>10,800 U/ml (52.1KU/g of substrate)</td>
<td>45.2%</td>
<td>50.0%</td>
<td></td>
</tr>
</tbody>
</table>

Method

Reaction condition:
- Buffer: 20 mM Tris HCl (pH 7.5)
- Reaction temperature: 30°C
- Unit definition: described in “Assay Method of Acylase Activity”

HPLC condition:
- Column: SUMICHIRAL OA-5000 (4.6 mm x 250 mm, Sumika Chemical Analysis Service, Ltd),
- Mobile phase: 1 mM Copper (II) sulfate in water-acetonitrile (95/5)
- Detector: UV, 260 nm
- Flow rate: 1 ml/min
Assay Method of Acylase Activity (DAC method)

**Reaction**

\[ N\text{-Acetyl-D,L-methionine} \xrightarrow{\text{D-Amino acylase}} \text{D-methionine} + \text{CH}_3\text{COOH} + N\text{-Acetyl-L-methionine} \]

D-Aminoacylase activity is measured at 570 nm by spectrophotometry.

**Procedure**

2 ml of Barbital buffer (B), 1 ml of cobalt solution (C), and 1 ml of Enzyme solution (G) are placed into a test tube. After pre-heating for 5 minutes, 1 ml of substrate solution (A) is added to the solution, mixed well, and kept at 37 ± 0.5°C for just 30 minutes. After zero minute (A₀) and 30 minutes (A₃₀), 1 ml of the reaction mixture are taken into a test tube and boiled in water-bath for 3 minutes. After cooling, 2 ml of Ninhydrin solution (D) are added and mixed well, and then 0.1 ml of Tin(II) chloride solution (E) are added and mixed well.

The test tubes are capped on a glass-ball and boiled in water-bath for just 20 minutes. After cooling, 10 ml of 1-propanol solution (F) are added and mixed well. The absorbance at 570 nm of the solution is measured.

**Calculation**

One unit is defined as enzyme quantity which produces one µmole of D-methionine per 30 minutes under the conditions described above.

\[
\text{D-Aminoacylase activity (U/g)} = \frac{(A_{30} - A_0)}{5 \times \text{Dm}} \times \frac{1}{149} \times 89.76
\]

- 89.76: Factor (Standard curve with D-methionine)
- 149: µg of 1µmole of D-methionine
- 5: Final volume of the reaction mixture
- 1: Volume of Enzyme Solution
- Dm: Dilution multiple of Enzyme Solution

**Remarks**

(A) Substrate solution: 0.1M N-Acetyl-D-methionine (pH8.0)
(B) Barbital buffer: 0.1M Barbital sodium-HCl buffer (pH 8.0)
(C) Cobalt solution: 0.0005M cobalt chloride solution
(D) Ninhydrin solution: Weigh 1 g of ninhydrin and dissolve in 25 ml of 2-methoxyethanol and fill up to 50 ml with 0.2M citric acid-NaOH buffer (pH5.0).
(E) Tin(II) chloride solution: Weigh 0.1 g of Tin(II) chloride dihydrate and dissolve in 6.2 ml 0.2M citric acid-NaOH buffer(pH5.0)
(F) 1-propanol solution: 1-Propanol/ deionized water (1/1)
(G) Enzyme solution: Weigh some of D-Amino acylase "Amano" and dissolve in 0.1M Barbital sodium-HCl buffer (pH 8.0)