SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of L-ALANINE DEHYDROGENASE
(EC 1.4.1.1)

PRINCIPLE:
L-Alanine + β-NAD + H₂O \( \rightarrow \) Pyruvate + β-NADH + NH₃

Abbreviations used:
- β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
- β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS:  \( T = 25°C, \ pH = 10.0, \ A_{340nm}, \ \text{Light Path} = 1 \text{ cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Bicarbonate Solution (NaHCO₃)
   (Prepare 100 ml in deionized water using Sodium Bicarbonate, Prod. No. S-8875.)

B. 50 mM Sodium Carbonate Solution (Na₂CO₃)
   (Prepare 150 ml in deionized water using Sodium Carbonate, Anhydrous, Prod. No. S-2127.)

C. 50 mM Sodium Carbonate Buffer, pH 10.0 at 25°C
   (Prepare 200 ml by adding 100 ml of Reagent B to 100 ml of Reagent A. Adjust to pH 10.0 at
   25°C with Reagent B.)

D. 500 mM L-Alanine Solution (L-ALA)
   (Prepare 1.0 ml in deionized water using L-Alanine, Prod. No. A-7627.)

E. 30 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form Solution (β-NAD)
   (Dissolve the contents of one 20 mg vial of β-Nicotinamide Adenine Dinucleotide, Stock No.
   260-120, in the appropriate volume of deionized water. PREPARE FRESH.)

F. L-Alanine Dehydrogenase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.2 - 0.5 unit/ml of L-Alanine
   Dehydrogenase in cold Reagent C.)
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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Buffer)</td>
<td>2.70</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent D (L-ALA)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (β-NAD)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
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<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Buffer)</td>
<td>-----</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>-----</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}
\]

3 = Volume (in milliliters) of assay  
df = Dilution factor  
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm  
0.1 = Volume (in milliliter) of enzyme used

\[
\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}
\]

\[
\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will convert 1.0 μmole of L-alanine to pyruvate and NH₃ per minute at pH 10.0 at 25°C.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 47 mM sodium bicarbonate, 17 mM L-alanine, 1.0 mM β-nicotinamide adenine dinucleotide and 0.02 - 0.05 unit of L-alanine dehydrogenase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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