SIGMA QUALITY CONTROL TEST PROCEDURE
Enzymatic Assay of L-AMINO ACID OXIDASE
(EC 1.4.3.2)

PRINCIPLE:

\[ \text{L-Phenylalanine} + \text{H}_2\text{O} \xrightarrow{\text{L-Amino Acid Oxidase, Catalase}} \text{Phenylpyruvate} \]

CONDITIONS: \( T = 37^\circ C, \ \text{pH} = 6.5, \ \text{A}_{308\text{nm}}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 200 mM Sodium Phosphate Buffer, pH 6.5 at 37°C
   (Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751. Adjust to pH 6.5 at 37°C with 1 M NaOH.)

B. 10 mM L-Phenylalanine Solution (L-Phe)
   (Prepare 10 ml in deionized water using L-Phenylalanine, Sigma Prod. No. P-2126.)

C. 2000 mM Sodium Arsenate Solution (Arsenate)
   (Prepare 20 ml in Reagent A using Arsenic Acid, Sodium Salt, Sigma Prod. No. A-6756.)

D. 2000 mM Boric Acid Solution, pH 6.5 at 37°C (Boric Acid)
   (Prepare 20 ml in Reagent C using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 6.5 at 37°C with 5 M HCl.)

E. Catalase Enzyme Solution (Catalase)
   (Immediately before use, prepare a solution containing 60,000 units/ml in cold deionized water using Catalase, Sigma Stock No. C-40.)

F. L-Amino Acid Oxidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of L-Amino Acid Oxidase in cold deionized water.)
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PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>11.70</td>
</tr>
<tr>
<td>Reagent B (L-Phe)</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent D (Boric Acid)</td>
<td>14.00</td>
</tr>
</tbody>
</table>

Mix by stirring and adjust to pH 6.5 at 37°C with 1 M HCl or 1 M NaOH, if necessary. 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>Reaction Cocktail</td>
<td>2.87</td>
<td>2.87</td>
</tr>
<tr>
<td>Reagent E (Catalase)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the $A_{308\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{308\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{308\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{308\text{nm}}/\text{min Test} - \Delta A_{308\text{nm}}/\text{min Blank}) (3) (df)}{(5.00) (0.1)}$$

$3 = \text{Total volume (in milliliters) of assay}$
$df = \text{Dilution factor}$
$5.00 = \text{Millimolar extinction coefficient of the phenylpyruvate keto borate complex at 308 nm}$
$0.1 = \text{Volume (in milliliter) of enzyme}$

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$
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PROCEDURE: (continued)

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

UNIT DEFINITION:

One unit will oxidatively deaminate 1.0 μmole of L-phenylalanine per minute at pH 6.5 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 171 mM sodium phosphate, 1.0 mM phenylalanine, 933 mM sodium arsenate, 933 mM boric acid, 1800 units catalase and 0.05 - 0.1 unit L-amino acid oxidase.

REFERENCE:


NOTES:

1. Catalase Unit Definition: One unit will decompose 1.0 μmole of H₂O₂ per minute at pH 7.0 at 25°C, while the H₂O₂ concentration falls from 10.3 to 9.2 mM.

2. This assay is based on the cited references.

3. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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