Enzymatic Assay of L-AMINO ACID OXIDASE  
(EC 1.4.3.2)

PRINCIPLE:

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

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

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, 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.)

C. 2000 mM Sodium Arsenate Solution (Arsenate)  
(Prepare 20 ml in Reagent A using Arsenic Acid, Sodium Salt.)

D. 2000 mM Boric Acid Solution, pH 6.5 at 37°C (Boric Acid)  
(Prepare 20 ml in Reagent C using Boric Acid. 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.)

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 A (Buffer)</th>
<th>11.70</th>
</tr>
</thead>
<tbody>
<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\textsubscript{308nm} 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\textsubscript{308nm} for approximately 10 minutes. Obtain the \( \text{r} \ A\textsubscript{308nm}/\text{minute} \) using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r \ A\textsubscript{308nm}/\text{min Test} - r \ A\textsubscript{308nm}/\text{min Blank}) (3) (df)}{(5.00) (0.1)}
\]

3 = Total volume (in milliliters) of assay

\( \text{df} \) = Dilution factor

5.00 = Millimolar extinction coefficient of the phenylpyruvate keto borate complex at 308 nm

0.1 = 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)

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

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.