Enzymatic Assay of PHENYLALANINE AMMONIA-LYASE
(EC 4.3.1.5)
L-Tyrosine as Substrate

PRINCIPLE:

\[
L-Tyrosine \xrightarrow{PAL} p-Coumarate + \text{NH}_4^+
\]

Abbreviation used:
PAL = Phenylalanine Ammonia-Lyase

CONDITIONS:  
T = 30°C, pH = 8.5, \(A_{286\text{nm}}\), Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 150 mM Tris HCl Buffer, pH 8.5 at 30°C
(Prepare 100 ml in deionized water using Trizma Base, Adjust to pH 8.5 at 30°C with 1 M HCl.)

B. 3 mM L-Tyrosine Solution (TYR)
(Prepare 25 ml in Reagent A using L-Tyrosine, Free Base. Heat gently to dissolve.)

C. Phenylalanine Ammonia-Lyase Enzyme Solution (PAL)
(Immediately before use, prepare a solution containing 0.5 - 1.0 unit/ml of Phenylalanine Ammonia-Lyase in cold Reagent A.)

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 B (TYR)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>0.95</td>
<td>0.95</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (PAL)</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>------</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{286\text{nm}}$ for approximately 5 minutes. Obtain the $r_{A_{286\text{nm}}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r_{A_{286\text{nm}}}/\text{min Test} - r_{A_{286\text{nm}}}/\text{min Blank})(3)(\text{df})}{(18.5)(0.05)(3)}$$

- $3 =$ Total volume (in milliliters) of assay
- $\text{df} =$ Dilution factor
- $18.5 =$ Millimolar extinction coefficient of p-coumaric acid$^1$
- at $286\text{nm}$
- $0.05 =$ 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 deaminate 1.0 µmole of L-tyrosine to p-coumarate and NH$_3$ per minute at pH 8.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 103 mM Tris, 2 mM L-tyrosine, and 0.025 - 0.050 unit phenylalanine ammonia-lyase.

REFERENCE:

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NOTES:

1. This assay is based on the cited reference.