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

PRINCIPLE:

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

Abbreviation used:  
PAL = Phenylalanine Ammonia-Lyase

CONDITIONS:  
T = 30°C, pH = 8.5, A_{286nm}, 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, Sigma Prod. No. T-1503. Adjust to pH 8.5 at 30°C with 1 M HCl.)

B. 3 mM \text{L-Tyrosine Solution (TYR)}  
(Prepare 25 ml in Reagent A using \text{L-Tyrosine, Free Base, Sigma Prod. No. T-3754. 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 = Total volume (in milliliters) of assay

$\text{df}$ = Dilution factor

18.5 = Millimolar extinction coefficient of p-coumaric acid at 286nm

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. The millimolar extinction coefficient was determined experimentally by Sigma.

2. This assay is based on the cited reference.

This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.