Enzymatic Assay of TYROSINASE  
(EC 1.14.18.1)

PRINCIPLE:

L-Tyrosine + O₂ → L-DOPA

L-DOPA → L-DOPA-quinone + H₂O

Abbreviation used:
L-DOPA = L-3,4-Dihydroxyphenylalanine

CONDITIONS:  T = 25°C, pH = 6.5, A₂₈₀nm, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  50 mM Potassium Phosphate Buffer, pH 6.5 at 25°C  
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.5 at 25°C with 1 M KOH.)

B.  1 mM L-Tyrosine Solution  
(Prepare 100 ml in deionized water using L-Tyrosine, Free Base, Sigma Prod. No. T-3754.)

C.  Tyrosinase Enzyme Solution  
(Immediately before use, prepare a solution containing 500 - 1,000 units/ml of Tyrosinase in cold Reagent A.)

PROCEDURE:

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

Deionized Water  9.00
Reagent A (Buffer)  10.00
Reagent B (Tyrosine)  10.00
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PROCEDURE: (continued)

Mix and adjust to pH 6.5 at 25°C with 1 M HCl or 1 M NaOH, if necessary. Immediately before use, oxygenate by bubbling 99.9% pure O₂ through the reaction cocktail for 3 to 5 minutes. Pipette (in milliliters) into suitable quartz cuvettes:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.90</td>
</tr>
</tbody>
</table>

Equilibrate to 25°C. Monitor the A₂₈₀nm until constant, using a suitably thermostatted spectrophotometer. Then add:

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

Immediately mix by inversion and record the increase in A₂₈₀nm for approximately 10 minutes. Obtain the r A₂₈₀nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r A₂₈₀nm/\text{min Test} - r A₂₈₀nm/\text{min Blank}) (df)}{(0.001) (0.1)}
\]

\[df = \text{Dilution factor}\]

\[0.001 = \text{The change in A}_₂₈₀\text{nm/minute per unit of Tyrosinase at pH 6.5 at 25°C in a 3 ml reaction mix}\]

\[0.1 = \text{Volume (in milliliters) of enzyme used}\]

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

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

UNIT DEFINITION:

One unit will cause an increase in A₂₈₀nm of 0.001 per minute at pH 6.5 at 25°C in a 3 ml reaction mix containing
L-tyrosine.
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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 18 mM potassium phosphate, 0.3 mM L-tyrosine and 50 - 100 units tyrosinase.

REFERENCE:


NOTES:

1. Final volume of all cuvettes must equal 3 ml as stated in the Unit Definition.

2. This assay is based on the cited reference.

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

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