Enzymatic Assay of TYROSINASE
Catechol Oxidase Activity
(EC 1.14.18.1)

PRINCIPLE:
Catechol + Ascorbic Acid $\xrightarrow{\text{Catechol Oxidase}}$ Dehydro-Ascorbic Acid + o-Benzoquinone

CONDITIONS:  $T = 25^\circ C$, $pH = 6.5$, $A_{265nm}$, Light path = 1 cm

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  50 mM Potassium Phosphate Buffer, $pH$ 6.5 at $25^\circ C$
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379.
Adjust to $pH$ 6.5 at $25^\circ C$ with 2 M NaOH.)

B.  5.0 mM Catechol Solution (Catechol)
(Prepare 10 ml in Reagent A using Catechol,
Prod. No. C-9510.)

C.  2.1 mM Ascorbic Acid Solution (Asc Acid)
(Prepare 10 ml in Reagent A using L-Ascorbic Acid,
Sodium Salt, Prod. No. A-7631. PREPARE FRESH.)

D.  0.065 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in Reagent A using Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Stock No. ED2SS.)

F.  Tyrosinase Enzyme Solution
(Immediately before use, prepare a solution containing 500 -1000 units/ml of Tyrosinase in Reagent A.)
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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.60</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent B (Catechol)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Asc Acid)</td>
<td>0.10</td>
<td>------</td>
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<tr>
<td>Reagent D (EDTA)</td>
<td>0.10</td>
<td>0.10</td>
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Mix by inversion and equilibrate to 25°C. Monitor the $A_{265nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

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<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
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Immediately mix by inversion and record the decrease in $A_{265nm}$ for approximately 5 minutes. Obtain the $rA_{265nm}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{rA_{265nm}/\text{min Test} - rA_{265nm}/\text{min Blank}}{(0.001) (\text{mg enzyme/RM})}$$

0.001 = The change in $A_{265nm}$ per unit of Tyrosinase in a 3.00 ml reaction mixture at pH 6.5 at 25°C.

RM = Reaction Mix

UNIT DEFINITION:

One unit is equal to a $rA_{265nm}$ of 0.001 per minute at pH 6.5 at 25°C in a 3 ml reaction mix containing catechol and L-ascorbic acid.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.17 mM catechol, 0.070 mM L-ascorbic acid, 0.0022 mM EDTA, and 50 - 100 units of catechol oxidase.
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REFERENCE:


NOTES:

1. This assay is a modification of the procedure cited in the above reference.

2. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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