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°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°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 6.5 at 25°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:

	Test	Blank
Reagent A (Buffer)	2.60	2.80
Reagent B (Catechol)	0.10	0.10
Reagent C (Asc Acid)	0.10	------
Reagent D (EDTA)	0.10	0.10

Mix by inversion and equilibrate to 25°C. Monitor the A_{265nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{265nm} for approximately 5 minutes. Obtain the $r\ A_{265nm}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

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 $r\ A_{265nm}$ 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:

Kandaswami, C. and Vaidyanathan, C.S. (1973) *Journal of Biological Chemistry* **248**, 4035.

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.