Enzymatic Assay of TYROSINASE
Polyphenol Oxidase Activity
(EC 1.14.18.1)

PRINCIPLE:

$$\text{L-DOPA} + O_2 + \text{Ascorbic Acid} \xrightarrow{\text{TYR}} \text{o-Benzoquinone} + H_2O + \text{Dehydro-Ascorbic Acid}$$

Abbreviations used:
TYR = Tyrosinase
L-DOPA = L-3,4-Dihydroxyphenylalanine

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 1 M NaOH.)

B. 5.0 mM L-3,4-Dihydroxyphenylalanine (L-DOPA)
(Prepare 10 ml in Reagent A using L-3,4-Dihydroxyphenylalanine, Prod. No. D-9628.)

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

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

E. Tyrosinase Enzyme Solution (PPO)
(Immediately before use, prepare a solution containing 500 - 1000 u/ml 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 (L-DOPA)	0.10	0.10
Reagent C (Ascorbic 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 E (TYR)	0.10	------

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

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(\Delta A_{265nm}/\text{min Test} - \Delta A_{265nm}/\text{min Blank})(df)}{(0.001)(0.1)}$$

0.001 = The change in A_{265nm}/min per unit of polyphenol oxidase in a 3.0 ml reaction mixture at pH 6.5 at 25°C.
0.1 = 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 is equal to a ΔA_{265nm} of 0.001 per min at pH 6.5 at 25°C in 3 ml reaction mix containing L-DOPA and L-ascorbic acid.

FINAL ASSAY CONCENTRATION:

In a 2.92 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.17 mM L-3,4-dihydroxyphenylalanine, 0.072 mM ascorbic acid, .0022 mM ethylenediaminetetraacetic acid, and 50 - 100 units of tyrosinase.

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NOTES:

1. 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.