Enzymatic Assay of TYROSINASE
(EC 1.14.18.1)

PRINCIPLE:

L-Tyrosine + O_2 $\xrightarrow{\text{Tyrosinase}}$ L-DOPA

L-DOPA $\xrightarrow{\text{Tyrosinase}}$ L-DOPA-quinone + H_20

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

CONDITIONS: T = 25°C, pH = 6.5, A_{280nm}, 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 O_2 through the reaction cocktail for 3 to 5 minutes. Pipette (in milliliters) into suitable quartz cuvettes:[1]

	Test	Blank
Reaction Cocktail	2.90	2.90

Equilibrate to 25°C. Monitor the A_{280nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent A (Buffer)	------	0.10
Reagent C (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{280nm} for approximately 10 minutes. Obtain the ΔA_{280nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{280nm}/\text{min Test} - \Delta A_{280nm}/\text{min Blank})\ (df)}{(0.001)\ (0.1)}$$

df = Dilution factor
0.001 = The change in A_{280nm}/minute per unit of Tyrosinase at pH 6.5 at 25°C in a 3 ml reaction mix
0.1 = Volume (in milliliters) 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 cause an increase in A_{280nm} 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:

Duckworth, H. W. and Coleman, J. E. (1970) *J. Biol. Chem.* **245**, 1613-1625

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.