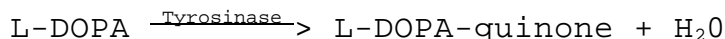
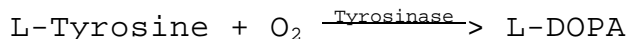


**Enzymatic Assay of TYROSINASE  
(EC 1.14.18.1)**

**PRINCIPLE:**



Abbreviation used:

L-DOPA = L-3,4-Dihydroxyphenylalanine

**CONDITIONS:** T = 25°C, pH = 6.5, A<sub>280nm</sub>, 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<sub>2</sub> through the reaction cocktail for 3 to 5 minutes. Pipette (in milliliters) into suitable quartz cuvettes:<sup>1</sup>

	<u>Test</u>	
	<u>Blank</u>	
Reaction Cocktail	2.90	2.90

Equilibrate to 25°C. Monitor the A<sub>280nm</sub> 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<sub>280nm</sub> for approximately 10 minutes. Obtain the r A<sub>280nm</sub>/minute using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

df = Dilution factor

0.001 = The change in A<sub>280nm</sub>/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<sub>280nm</sub> 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.

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