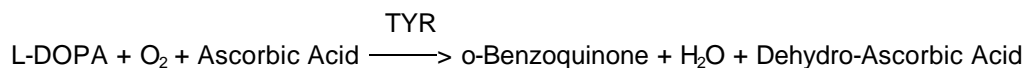


**Enzymatic Assay of TYROSINASE  
Polyphenol Oxidase Activity  
(EC 1.14.18.1)**

**PRINCIPLE:**



Abbreviations used:

TYR = Tyrosinase

L-DOPA = L-3,4-Dihydroxyphenylalanine

**CONDITIONS:** T = 25°C, pH 6.5,  $A_{265\text{nm}}$ , 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:

	<u>Test</u>	<u>Blank</u>
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  $A_{265\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (TYR)	0.10	-----
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Immediately mix by inversion and record the decrease in  $A_{265\text{nm}}$  for approximately 5 minutes. Obtain the  $\Delta A_{265\text{nm}}/\text{minute}$  using the maximum linear rate for both the Test and Blank.

**CALCULATIONS:**

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

0.001 = The change in  $A_{265\text{nm}}/\text{min}$  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  $\Delta A_{265\text{nm}}$  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.**