Enzymatic Assay of POLYPHENOL OXIDASE
(EC 1.14.18.1)

PRINCIPLE:

$_{L}$-DOPA + O_2 $\xrightarrow{PPO}$ Benzoquinone derivative + H_2O

Benzoquinone derivative + $_{L}$-Asc Acid ——> $_{L}$-DOPA + Dehydro-Asc Acid

Abbreviations:
$_{L}$-DOPA = $_{L}$-3,4-Dihydroxyphenylalanine
PPO = Polyphenol Oxidase
$_{L}$-Asc = $_{L}$-Ascorbic Acid
Dehydro-Asc Acid =Dehydro-Ascorbic Acid

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, Prod. No. P-5379. Adjust to pH 6.5 at 25°C with 1 M NaOH.)

B. 5 mM $_{L}$-3,4-Dihydroxyphenylalanine Solution ($_{L}$-DOPA)
(Prepare 10 ml in Reagent A using $_{L}$-3,4-Dihydroxyphenylalanine, Prod. No. D-9628. **PREPARE FRESH.**)

C. 2.1 mM $_{L}$-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 Solution (EDTA)
(Prepare 10 ml in Reagent A using Ethylenediaminetetraacetic Acid, Disodium Salt, Stock No. ED2SS.)

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REAGENTS: (continued)

E. Polyphenol Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 500 - 1000 units/ml of Polyphenol Oxidase in cold Reagent A.)

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	------[1]
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 (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the decrease in A_{265nm} for approximately 5 minutes. Obtain the $r\ A_{265nm}$/minute 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}/minute per unit Polyphenol Oxidase at pH 6.5 at 25°C in a 3 ml reaction mix

RM = Reaction Mix (final volume = 3 ml)

UNIT DEFINITION:

One unit will cause the change in A_{265nm} of 0.001 per minute at pH 6.5 at 25°C in a 3 ml reaction mix containing L-3,4-dihydroxyphenylalanine and L-ascorbic acid.

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FINAL ASSAY CONCENTRATION:

In a 3 ml reaction mix, the final concentrations are 50 mM potassium phosphate, 0.17 mM L-3,4-dihydroxyphenylalanine, 0.07 mM L-ascorbic acid and 50 - 100 units of polyphenol oxidase.

REFERENCES:

Dawson, C.R., and Magee, R.J. (1955) *Methods in Enzymology* **II**, 817-821.

Marumo, K., and Waite, J.H. (1986) *Biochim. Biophys. Acta* **872**, 98-103.

NOTES:

1. Ascorbic acid is not included in the blank to produce a true blank. Possible interaction with L-Dopa and ascorbic acid might give false readings.

2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.