

**Enzymatic Assay of POLYPHENOL OXIDASE
(EC 1.14.18.1)**

PRINCIPLE:

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

$\text{Benzoquinone derivative} + L\text{-Asc Acid} \longrightarrow L\text{-DOPA} + \text{Dehydro-Asc Acid}$

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.)

**Enzymatic Assay of POLYPHENOL OXIDASE
(EC 1.14.18.1)**

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:

	<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	----- 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:

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

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.

**Enzymatic Assay of POLYPHENOL OXIDASE
(EC 1.14.18.1)**

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.

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