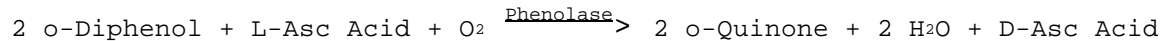


**Enzymatic Assay of PHENOLASE
(EC 1.10.3.1)**

PRINCIPLE:



Abbreviations:

Asc Acid = L-Ascorbic Acid

D-Asc Acid = Dehydro-Ascorbic Acid

CONDITIONS: T = 30°C, pH = 7.5, A_{265nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Potassium Phosphate Buffer, pH 7.5 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Prod. No. P-5379. Adjust to pH 7.5 at 30°C with 1 M KOH.)
- B. 10 mM Chlorogenic Acid Solution (Chloro)
(Prepare 10 ml in Reagent A using Chlorogenic Acid, Prod. No. C-3878. **PREPARE FRESH.**)
- C. 1 mM L-Ascorbic Acid Solution (Asc)
(Prepare 10 ml in Reagent A using L-Ascorbic Acid, Free Acid, Prod. No. A-7506. **PREPARE FRESH.**)
- D. 100 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Stock No. ED4SS.)
- E. Phenolase Enzyme Solution
(Immediately before use, prepare a solution containing 200 - 300 units/ml of Phenolase in cold Reagent A.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable quartz cuvettes:

	<u>Test</u>	<u>Blank</u>
Deionized Water	0.67	0.67
Reagent A (Buffer)	2.09	2.10
Reagent B (Chloro)	0.10	0.10
Reagent C (Asc)	0.10	0.10
Reagent D (EDTA)	0.03	
		0.03

Mix by inversion and equilibrate to 30°C. Monitor the $A_{265\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent E (Enzyme Solution)	0.01	-----
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Immediately mix by inversion and record the decrease in $A_{265\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{265\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{(r A_{265\text{nm}}/\text{min Test} - r A_{265\text{nm}}/\text{min Blank})}{(0.01) (\text{mg enzyme/RM})}$$

0.01 = The change in $A_{265\text{nm}}/\text{minute}$ per unit Phenolase at
pH 7.5 at 30°C in a 3 ml reaction mix
RM = Reaction Mix

UNIT DEFINITION:

One unit will produce a $r A_{265\text{nm}}$ of 0.01 per minute at pH 7.5 at 30°C, using chlorogenic acid as substrate in a 3 ml reaction volume.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 38 mM potassium phosphate, 0.33 mM chlorogenic acid, 0.03 mM L-ascorbic acid, 1 mM EDTA and 2.0 - 3.0 units of phenolase.

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(EC 1.10.3.1)**

REFERENCES:

Patil, S.S. and Zuker, M. (1965) *J. Biol. Chem.* **240**, 3938.

NOTES:

1. The blank will have an absorbance of approximately 2.1.
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.