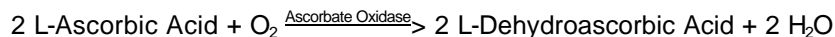


**Enzymatic Assay of ASCORBATE OXIDASE
(EC 1.10.3.3)**

PRINCIPLE:



CONDITIONS: T = 25°C, pH = 5.6, A_{245nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Potassium Phosphate, and 4 mM Sodium Phosphate Buffer with 0.5 mM Ethylenediaminetetraacetic (EDTA), pH 5.6 at 25°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Sigma Prod. No. P-5379; Sodium Phosphate, Dibasic, Sigma Prod. No. S-0876; and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.6 with 1 M HCl or 1 M KOH at 25°C if necessary.)
- B. 200 mM Hydrochloric Acid (HCl)
(Prepare 100 ml in deionized water using Hydrochloric Acid, Sigma Prod. No. H-7020.)
- C. 0.5 mM L-Ascorbic Acid Solution (Ascorbate Substrate)
(Prepare 50 ml in Reagent A using L-Ascorbic Acid, Free Acid, Sigma Prod. No. A-7506; **PREPARE FRESH.** To standardize, mix 1 ml of 0.5 mM L-Ascorbic Acid with 3 ml of Reagent B immediately before measurement and read the absorbance at 245 nm against deionized water. The $A_{245\text{nm}}$ should be 1.25 ± 0.05. If the absorbance reading is not in this range, dilute the solution with Reagent A or with 0.5 mM L-Ascorbic Acid until the $A_{245\text{nm}}$ equals 1.25 ± 0.05.)
- D. 4 mM Sodium Phosphate Buffer with 0.05% (w/v) Bovine Serum Albumin, pH 5.6 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Sigma Prod. No. S-0876 and Albumin, Bovine, Sigma Prod. No. A-6003. Adjust the pH to 5.6 at 25°C with 1 M HCl.)

**Enzymatic Assay of ASCORBATE OXIDASE
(EC 1.10.3.3)**

REAGENTS: (continued)

- E. Ascorbate Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.12 - 0.24 unit/ml of Ascorbate Oxidase in cold Reagent D.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable vials:

	<u>Test</u>	<u>Blank</u>
Reagent C (Ascorbate Substrate)	1.00	1.00

Equilibrate for several minutes at 25°C. Then add:

Reagent E (Enzyme Solution)	0.10	-----
-----------------------------	------	-------

Mix by swirling and incubate at 25°C for exactly 5 minutes. Then add:

Reagent B (HCl)	3.00	3.00
-----------------	------	------

Mix by swirling and then add:

Reagent E (Enzyme Solution)	-----	0.10
-----------------------------	-------	------

Mix by swirling. Transfer the solutions to suitable cuvettes and record the $A_{245\text{nm}}$ of the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{245\text{nm}} \text{ Blank} - A_{245\text{nm}} \text{ Test})(4.1)(\text{df})}{(10)(5)(0.1)}$$

5 = Time (in minutes) of assay as per the Unit Definition

10 = Millimolar extinction coefficient of L-Ascorbic Acid at 245 nm under the assay conditions

0.1 = Volume (in milliliter) of enzyme used

4.1 = Total volume (in milliliters) of assay

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

**Enzymatic Assay of ASCORBATE OXIDASE
(EC 1.10.3.3)**

CALCULATIONS: (continued)

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 μ mole of L-ascorbate to dehydroascorbate per minute at pH 5.6 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 91 mM potassium phosphate, 0.45 mM L-ascorbic acid, 0.5 mM ethylenediaminetetraacetic acid, 4 mM sodium phosphate, 0.005% (w/v) bovine serum albumin, and 0.012 - 0.024 unit of ascorbate oxidase.

REFERENCE:

Bergmeyer, H.U., Grassl, M. and Walter, H.E. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 3rd ed., Volume II, 157-158, Verlag Chemie, Deerfield Beach, FL

NOTES:

1. This assay is based on the cited reference.
2. 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.