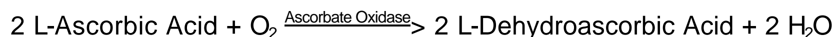
SIGMA
3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of ASCORBATE OXIDASE
(EC 1.10.3.3)

PRINCIPLE:



2 L-Ascorbic Acid + O_2 $\xrightarrow{\text{Ascorbate Oxidase}}$ 2 L-Dehydroascorbic Acid + 2 H_2O

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_{245nm} 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_{245nm} 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:

	Test	Blank
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_{245nm} of the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

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

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

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

CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole 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.

Sigma warrants that the above procedure information is currently utilized at Sigma and that Sigma products conform to the information in Sigma publications. Purchaser must determine the suitability of the information and products for its particular use. Upon purchase of Sigma products, see reverse side of invoice or packing slip for additional terms and conditions of sale.