Ascorbate Oxidase“Amano”3

(L-Ascorbate : oxygen oxidoreductase, EC 1.10.3.3)

Ascorbate Oxidase“Amano”3 is an ascorbate oxidase preparation, manufactured using recombinant filamentous fungi.

Catalysis

L-Ascorbic acid + O_2 ⟶ L-Dehydroascorbic acid + H_2O_2

Specification and Preparation

Activity: Ascorbate oxidase activity ≧ 200 u/mg (Amano method)
Appearance: Light yellowish white to light yellow powder, lyophilized
Additive: Lactose

Characteristics

1. Molecular weight: 130,000 (Gel filtration)
2. Isoelectric point: 9.0
3. Km: 4.9×10^{-4} M (L-Ascorbic acid)
4. Optimum pH: 6.0
5. pH stability: 3.0-8.0 (37°C, 16hrs)
6. Optimum temperature: 37°C
7. Thermal stability: up to 50°C (pH 6.8, 16hrs)
8. Inhibitors: Fe^{2+}, Fe^{3+}, Monoiodoacetate, *N*-Ethylmaleimide
9. Application: Used for the enzymatic determination of ascorbic acid and for eliminating the interfere of ascorbic acid in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

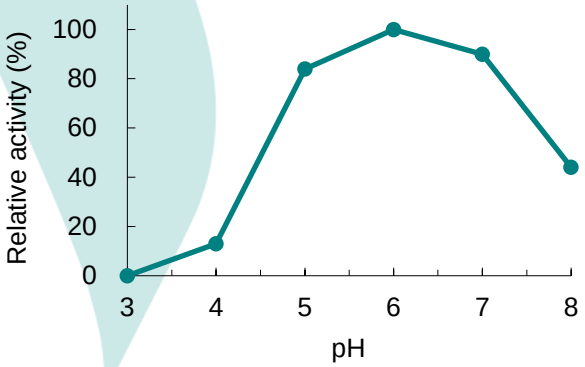
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

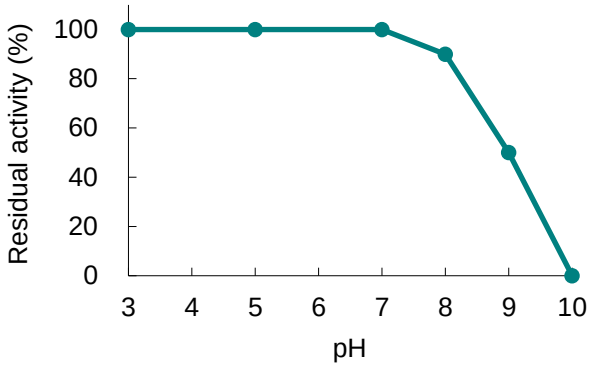
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

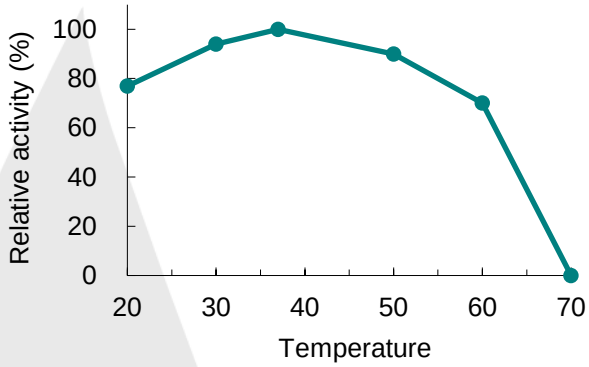
pH and Activity



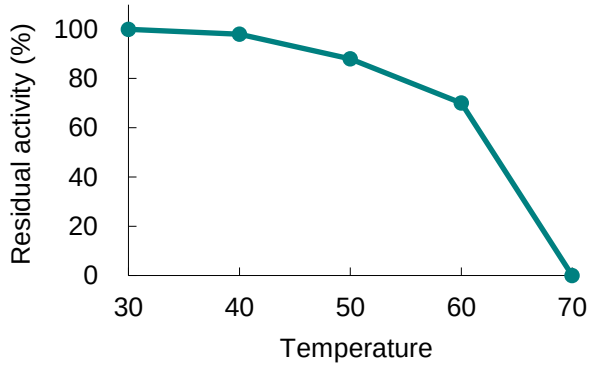
pH stability



Temperature and Activity



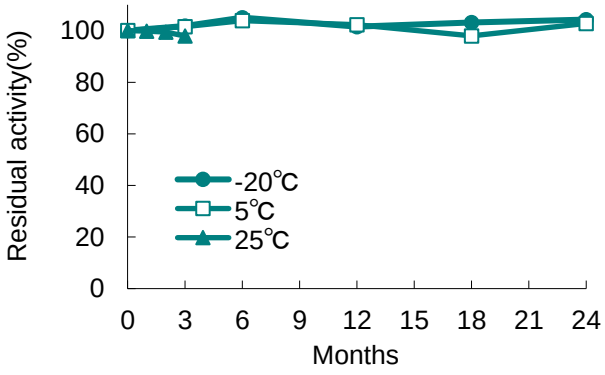
Thermostability



Substrate Specificity

Substrate	Relative Activity (%)
L-Ascorbic acid	100
D-Araboascorbic acid	0
Pyrogallol	0
Hydroquinone	0

Stability (powder form)



Effect of Various Chemicals

Chemicals	Concentration (mM)	Relative Activity (%)	Chemicals	Concentration (mM)	Relative Activity (%)
None	—	100	$CuCl_2$	5	100
NaCl	100	98	NaN_3	10	107
$MgCl_2$	5	98	EDTA	10	91
$CaCl_2$	5	102	α, α'-Dipyridyl	10	101
$MnCl_2$	5	98	o-Phenanthroline	10	105
$ZnCl_2$	5	100	8-Hydroxyquinoline	10	92
$CoCl_2$	5	96	Thiourea	10	105
$FeCl_2$	5	68	Diethyldithiocarbamate	10	101
$FeCl_3$	5	7	Dithiothreitol	10	99
$NiCl_2$	5	101	Monoiodoacetate	10	52
$BaCl_2$	5	98	*N*-Ethylmaleimide	10	88

Assay method of Ascorbate oxidase activity

Principle

$$\text{L-Ascorbic acid} + O_2 \xrightarrow{\text{Ascorbate oxidase}} \text{L-Dehydroascorbic acid} + H_2O_2$$

The consumption of Ascorbic acid is measured at 245 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of L-Ascorbic acid per minute under the conditions described as follows.

Reagents

A. 0.2 M HCl solution
B. 1 mM HCl solution (containing 1mM EDTA)
C. 10 mM Na_2HPO_4 solution
D. 0.2M KH_2PO_4 solution (containing 1mM EDTA)
E. 10 mM KH_2PO_4 solution (containing 0.05% BSA)
   Weigh 1.36 g of KH_2PO_4 and 0.50 g of bovine serum albumin (Low salt, FUJIFILM Wako Pure Chemical Corporation), then dissolve in approx. 500 ml of deionized water. Fill up to 1000 ml with deionized water. (Can be used for 14 days if kept refrigerated)
F. Substrate stock solution
   Weigh 88.0 mg of L-ascorbic acid and dissolve in approx. 40 ml of 1 mM HCl solution (B). Fill up to 50 ml with 1 mM HCl solution (B). (Can be used for 1 month if stored refrigerated and away from light)
G. Substrate solution
   Accurately measure out 5 ml of Substrate stock solution (F), dilute with 0.2 M KH_2PO_4 solution (D), and fill up to 50 ml. (Must store refrigerated and away from light, use within 1 hour after preparation)
H. Enzyme solution
   Weigh out Ascorbate Oxidase"Amano"3 and dissolve in chilled 10 mM KH_2PO_4 solution (E). Enzyme solution should be prepared so that the value of (A0-A5) becomes in the range of 0.2±0.10. (Use it within 3 hours after preparation)

Procedure

Pipette 0.5ml of Substrate solution (G) and 0.5 ml of 10 mM Na_2HPO_4 solution (C) respectively into a test tube and mix, then keep at 37±0.5℃ for 5 minutes in a water bath. After this pre incubation, pipette 0.1 ml of Enzyme solution (H) into the test tube and mix well, then keep at 37±0.5℃. At exactly 5 minutes, pipette 3 ml of 0.2 M HCl (A) and mix well to stop the reaction. Measure the absorbance of the reaction mixture at 245 nm (A5).
As a blank, pipette 0.5 ml of Substrate solution (G) and 0.5 ml of 10 mM Na_2HPO_4 solution (C) into another test tube respectively and mix, keep at 37±0.5℃ for 10 minutes. Then, pipette 3 ml of 0.2 M HCl (A) and mix well.
Pipette 0.1 ml of Enzyme solution (H) into the mixture and measure the absorbance at 245 nm (A0).

It is recommended that the enzyme reaction and blank process be carried out at the same time to prevent variation in measured values.

Calculation

$$\text{Ascorbate oxidse activity (u/mg)} = \frac{(A0-A5)}{5} \times \frac{1}{10.0} \times 4.1 \times \frac{n}{0.1}$$

5: Reaction time
10.0: Millimolar absorption coefficient of L-ascorbic acid at 245 nm
4.1: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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