L-alpha-Glycerophosphate Oxidase“Amano”3

(*Sn*-Glycerol-3-phosphate : oxygen 2-oxidoreductase, EC 1.1.3.21)

L-alpha-Glycerophosphate Oxidase“Amano”3 is a L-α-glycerophosphate oxidase preparation manufactured using a recombinant bacteria.

Catalysis

L-α-Glycerophosphate + O_2 ⟶ Dihydroxyacetone phosphate + H_2O_2

Specification and Preparation

Activity:	L-α-Glycerophosphate oxidase activity	≧ 35 u/mg (Amano method)
Contaminants:	Lactase oxidase activity	≦ 3×10^{-3}%
	Catalase activity	≦ 4×10^{-3}%
	ATPase activivity	≦ 6×10^{-3}%

Appearance: Yellow powder, lyophilized
Additive: Not added

Characteristics

1. Molecular weight: 67,000 (SDS-PAGE)
2. Isoelectric point: 4.4
3. Km: 1.18×10^{-4} M (pH 7.0, 37°C)
4. Optimum pH: 7.0
5. pH stability: 5.0-7.0 (37°C, 30 min)
6. Optimum temperature: 45°C
7. Thermal stability: up to 40°C (pH 7.0, 10 min)
8. Inhibitors: Ag^+, Hg^{2+}
9. Stabilizers: FAD, $(NH_4)_2SO_4$
10. Application: Used for the enzymatic determination of triglyceride in serum by coupling with lipoprotein lipase and glycerol kinase in clinical diagnosis.

Expiration (Storage)

24 months from the date of analysis when stored at 5°C or below in a dry place under sealed conditions.

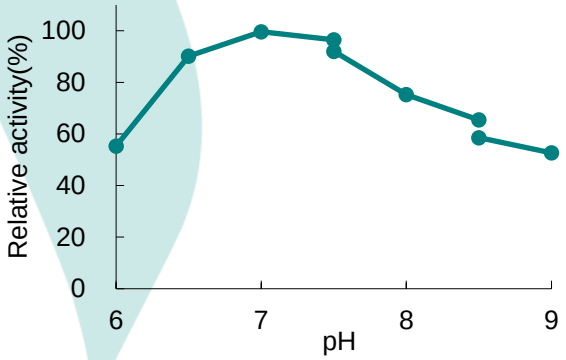
Safe Handling

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.

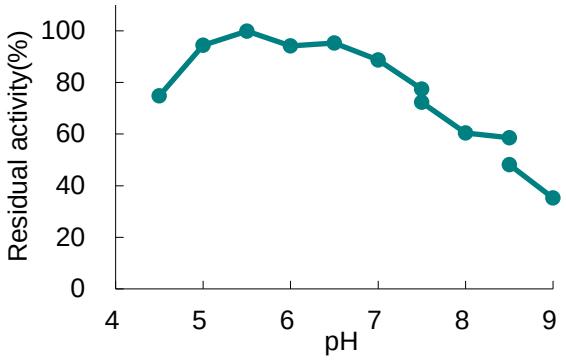
General properties

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

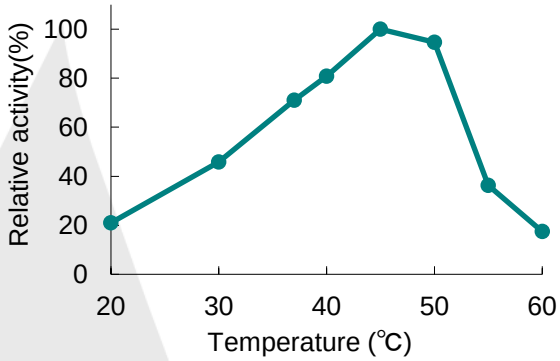
pH and Activity



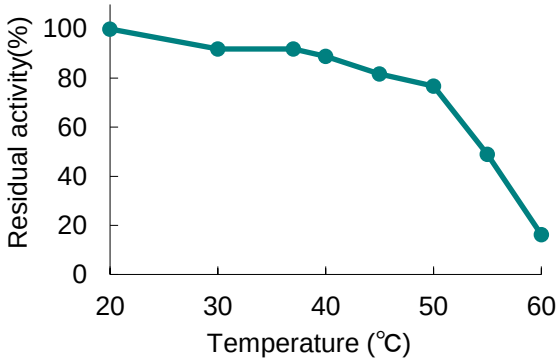
pH stability



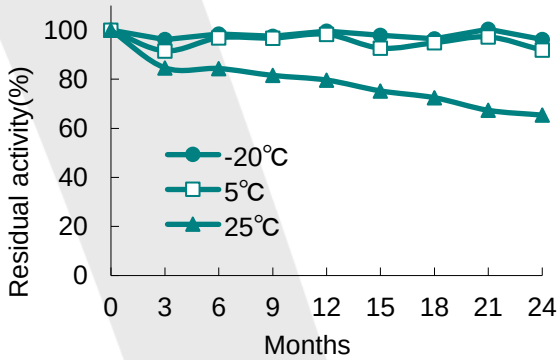
Temperature and Activity



Thermostability



Stability (powder form)



Assay method of L-α-Glycerophosphate oxidase activity

Principle

$$\text{L-α-Glycerophosphate} + O_2 \xrightarrow{\text{L-α-Glycerophosphate Oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

The appearance of quinoneimine dye formed by coupling with 4-aminoantipyrine and phenol is measured at 505 nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of L-α-glycerophosphate per minute under the conditions described below.

Reagents

A. 4-Aminoantipyrine solution (5 mg/ml deionized water)

B. Phenol solution (50mg/ml deionized water)

C. 0.2M Phosphate buffer (KH_2PO_4-NaOH, pH7.0)

D. 0.02M Phosphate buffer (KH_2PO_4-NaOH, pH7.0)

E. Peroxidase solution

Weigh 1000 units of Peroxidase (Amano Enzyme) and dissolved in 5ml of chilled 0.2M Phosphate buffer (C).

F. Aminoantipyrine-phenol solution

Mix approx. 30 ml of chilled 0.2M Phosphate buffer (C), 1 ml of Peroxidase solution (E), 6ml of 4-Aminoantipyrine solution (A) and 0.5 ml of Phenol solution (B), then fill up to 50 ml with chilled 0.2M Phosphate buffer (C). (Can be used for 14 days in light-shielded and refrigerated storage)

G. Substrate solution

Weigh 36.7 g of disodium (+/-)-1-glycerophosphate n-hydrate (FUJIFILM Wako Pure Chemical Corporation) and dissolve in approx. 40ml of deionized water. Adjust the pH to 7.0 with 4N HCl and fill up to 100 ml with deionized water. Then, filtrate the solution with 0.45μm membrane filter. (Can be used 14 days if kept refrigerated)

H. Diluent

Weigh 66.0 g of ammonium sulfate and dissolved in approx. 800 ml of 0.02M Phosphate buffer (D). Add 1.0 g of bovine serum albumin and dissolve, then fill up to 1000 ml with 0.02M Phosphate buffer (D).

I. Enzyme solution

Weigh out L-alpha-Glycerophosphate Oxidase“Amano”3 and dissolve in diluent (H).

Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.020 ± 0.005.

Procedure

Pipette 1.5 ml of Aminoantipyrine-phenol solution (F), 1.5 ml of Substrate Solution (G) respectively into a quartz cell (d=10 mm) and keep at 37±0.5°C for 10 minutes. Then, pipette 0.1 ml of Enzyme solution (I) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5°C. Exactly at 1 minute and 3 minutes after the addition of Enzyme Solution (I), measure the absorbances of the reaction mixture at 505 nm (A1 and A3). As a blank, pipette Diluent (H) into another quartz cell (d= 10 mm) instead of Enzyme solution (I) and take the same procedure described above (Ab1 and Ab3).

Calculation

$$\text{L-α-Glycerophosphate oxidase activity (u/mg)} = \frac{(A3-A1)-(Ab3-Ab1)}{2} \times \frac{1}{13.2} \times 2 \times 3.1 \times \frac{n}{0.1}$$

2: Reaction time
13.2: Millimolar absorption coefficient of quinoneimine dye
2: Conversion factor (1 mole of quinoneimine dye corresponds to 2 mole of glycerophosphate)
3.1: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

Contact

Area	Branch	Location	E-mail
North, Central, South America	Amano Enzyme U.S.A. Co., Ltd.	Illinois,U.S.A.	aeu.sales@amano-enzyme.com
Europe, the Middle East and Africa	Amano Enzyme Europe Limited	Oxfordshire, U.K.	aee.sales@amano-enzyme.com
Asia Pacific	Amano Enzyme Asia Pacific Co., Ltd.	Pathum Thani, THAILAND	aeap.sales@amano-enzyme.com
China	Amano Enzyme Manufacturing (China), Ltd. Shanghai Branch	Shanghai, P.R.CHINA	shanghai@amano-enzyme.com.cn
Japan, Headquarters	Amano Enzyme Inc.	Nagoya, JAPAN	