Esterase“Amano”2

(Sterol esterase, EC 3.1.1.13)

Esterase“Amano”2 is a sterol esterase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

Steryl ester + H_2O ⇄ Sterol + Fatty acid

Specification and Preparation

Activity:	Esterase activity	≧ 3.00 u/mg (Amano method)
Appearance:	White to light brown powder, lyophilized	
Additive:	BSA	

Characteristics

1. Molecular weight: 40,000 Da (SDS-PAGE)
2. pH stability: 5.0 - 10.0 (37°C, 1hr)
3. Optimum temperature: 40°C
4. Thermal stability: up to 50°C (pH 7.0, 1hr)
5. Activators: Triton X-100, Sodium cholate
6. Inhibitors: Cu^{2+}, *N*-Ethylmaleimide
7. Application: Used for the enzymatic determination of cholesterol in serum by coupling with cholesterol oxidase or cholesterol dehydrogenase 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.

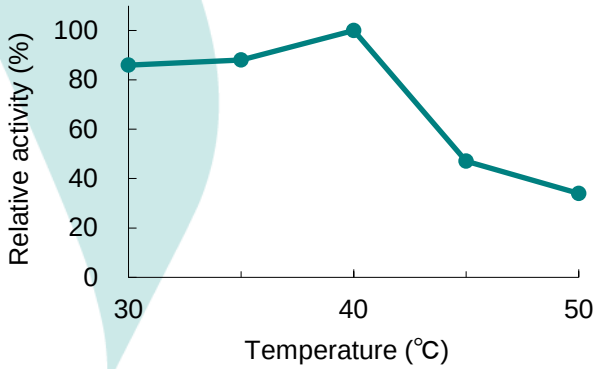
Safe Handling

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.

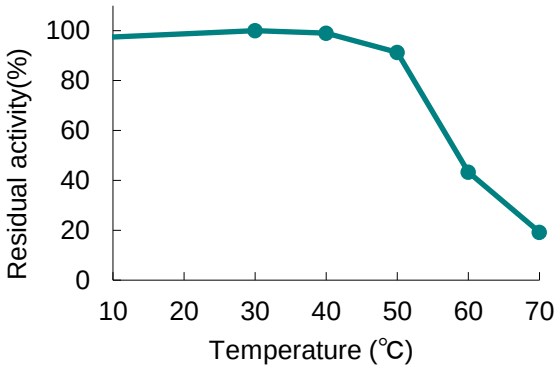
General properties

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

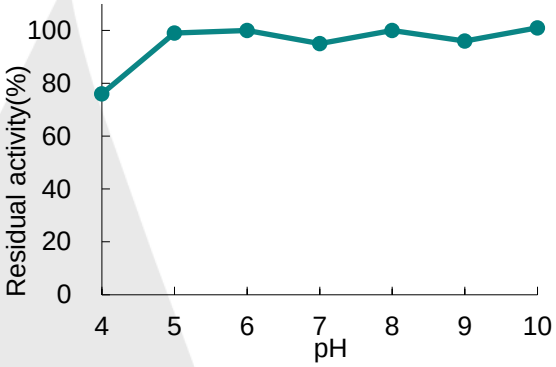
Temperature and Activity



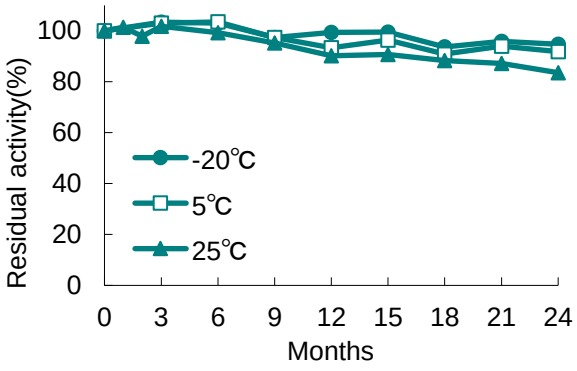
Thermostability



pH stability



Stability (powder form)

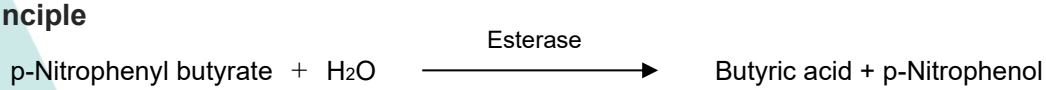


Effect of Various Chemicals

Chemicals (1mM)	Residual Activity (%)	Chemicals (1mM)	Residual Activity (%)
None	100	Diethyldithiocarbamate	105
$CaCl_2$	102	Thiourea	98
$MgCl_2$	106	α,α'-Dipyridyl	107
$CoCl_2$	97	o-Phenanthroline	104
$BaCl_2$	106	8-Hydroquinone	109
$MnCl_2$	110	*N*-Ethylmaleimide	70
$ZnCl_2$	95	Monoiodoacetic acid	96
$CuCl_2$	86	Triton X-100	253
$NiSO_4$	99	Sodium cholate	123
EDTA	102		

Assay method of Esterase activity

Principle



The appearance of p-Nitrophenol is measured at 410nm by spectrophotometry.

Unit Definition

One unit is defined as the enzyme quantity which produces 1 μmol of p-Nitrophenol per minute under the conditions described below.

Reagents

A. Triton X-100 solution（4 % Triton X-100 solution）
Weigh 4.0 g of Triton X-100 and dissolve in approx. 40 mL of deionized water with heating. After cooling, fill up to 100 mL with deionized water. (Can be used for 1 month at room temperature)

B. 0.1 M Phosphate buffer (KH_2PO_4-NaOH (pH 7.0))

C. 0.01 M Phosphate buffer (KH_2PO_4-NaOH (pH 7.0))

D. Substrate solution
Weigh 20.9 mg of p-Nitrophenyl butyrate (Sigma-Aldrich No. N9876), add 20 mL of Triton X-100 solution (A), and dissolve by stirring while heating at 60 ± 5°C for 15 minutes. After cooling, add 4 mL of 0.1 M Phosphate buffer (B) and 16 mL of deionized water. (Make a fresh solution for each use.)

E. Enzyme solution
Weigh out Esterase “Amano” 2 and dissolve in chilled 0.01M Phosphate buffer (C). Enzyme solution should be prepared so that the value of ΔOD/2minutes becomes in the range of 0.10-0.20/2min.

Procedure

Pipette 3.0 mL of Substrate solution (D) into a quartz cell (d=10 m) and keep at 37±0.5 ℃ for 5 minutes. Then, pipette 0.1 mL of Enzyme solution (E) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5 ℃. Exactly at 1 minute and 3 minutes after the addition of Enzyme Solution (E), measure the absorbances of the reaction mixture at 410 nm. (A1 and A3). As a blank, pipette Enzyme diluent (C) into another quartz cell (d=10 mm) instead of Enzyme Solution (E), and take the same procedure described above. (Ab1 and Ab3)

Calculation

$$\text{Esterase activity (u/mg)} = \frac{(A3-A1)-(Ab3-Ab1)}{2} \times \frac{1}{6.45} \times 3.1 \times \frac{n}{0.1}$$

2: Reaction time
6.45: Millimolar absorption coefficient of p-Nitrophenol at 410 nm
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	