Cholesterol Esterase “Amano” 2A

(Steryl-ester acylhydrolase, EC 3.1.1.13)

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

Catalysis

Cholesterol ester + H_2O ⟶ Cholesterol + Fatty acid

Specification and Preparation

Activity:	Cholesterol esterase activity	≧ 10 u/mg (Amano method)
Contaminant:	Catalase activity	≦ 5%
Appearance:	Light yellowish white to light brown powder, lyophilized	
Additive:	BSA	

Characteristics

1. Molecular weight: 30,000 (SDS-PAGE)
2. Isoelectric point: 6.0
3. Km: 3×10^{-5} M
4. Optimum pH: 7.0
5. pH stability: 5.0-11.0 (37°C, 1hr)
6. Optimum temperature: 35°C
7. Thermal stability: up to 60°C (pH 7.0, 10 min)
8. Activator: Triton X-100
9. Inhibitors: Ag^{+}, Hg^{2+}
10. Stabilizers: Mg^{2+}, Ca^{2+}
11. Application: Used for the enzymatic determination of cholesterol in serum by coupling with cholesterol oxidase or cholesterol dehydrogenase in clinical diagnosis.
12. Note: Product may include 4-7% of 4-(1,1,3,3-Tetramethylbutyl)phenyl, Ethoxylated which is included in the Authorisation List of Substances of Very High Concern (SVHC) in the Annex XIV of Regulation (EC) No. 1907/2006 (REACH).

Expiration (Storage)

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

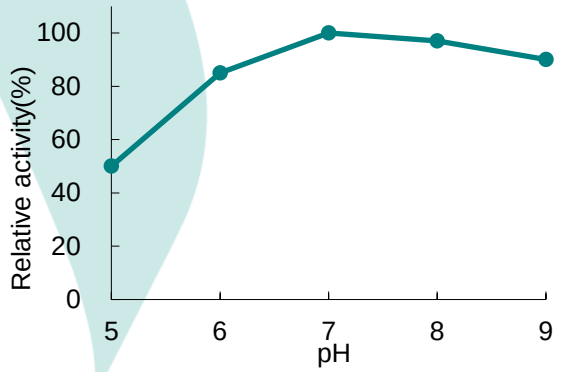
Safe Handling

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.

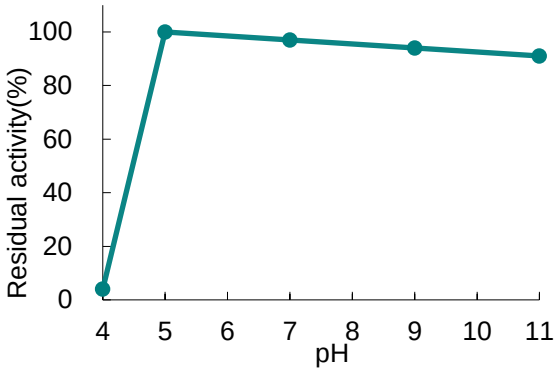
General properties

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

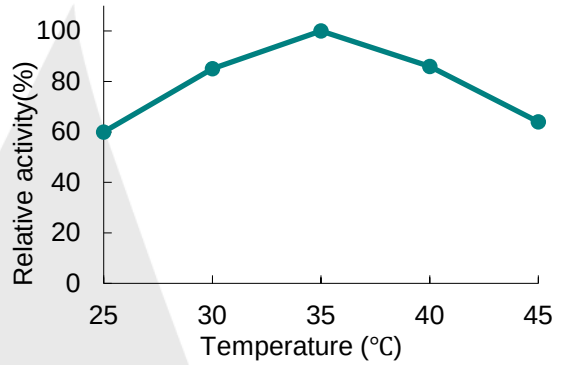
pH and Activity



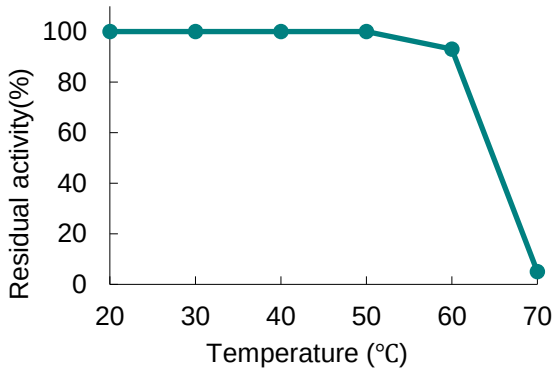
pH stability



Temperature and Activity



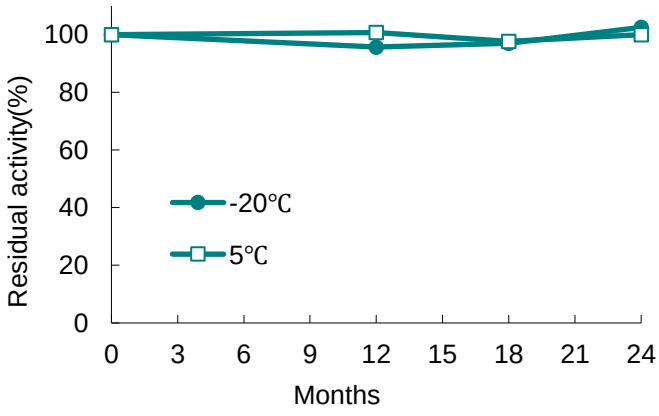
Thermostability



Substrate Specificity

Substrate	Relative Activity (%)
Cholesterol acetate	6
Cholesterol butyrate	9
Cholesterol caprinate	28
Cholesterol laurinate	80
Cholesterol myristate	84
Cholesterol palmitate	94
Cholesterol oleate	90
Cholesterol linoleate	100
Testosterone	0

Stability (powder form)



Assay method of Cholesterol esterase activity

Principle

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol Esterase}}$ Cholesterol + RCOOH

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ Cholest-4-en-3-one + H_2O_2

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

The appearance of quinoneimine dye formed by coupling with 4-aminoantipyrine and phenol is measured at 500nm by spectrophotometry.

Unit Definitio

One unit is defined as the enzyme quantity which hydrolyzes one μ mole of cholesterol ester per minute under the conditions described below.

Reagents

A. 4-Aminoantipyrine solution (5 mg/ml deionized water)
B. Triton X-100 solution (50 mg/ml deionized water)
C. Phenol solution (50mg/ml deionized water)
D. 0.15M Phosphate buffer (KH_2PO_4-KOH, pH7.0)
E. Aminoantipyrine-phenol solution
Weigh 2.04g of KH_2PO_4 and dissolve in 70ml of deionized water. Add 6ml of 4-Aminoantipyrine solution (A) and 2ml of Phenol solution (C), then adjust the pH to 7.0 with 1N KOH. Add 300 units of Peroxidase (Amano Enzyme) and 50 units of Cholesterol Oxidase (Amano Enzyme) to the solution and fill up to 100ml with deionized water. (Can be used for 12 days in light-shielded and refrigerated storage)
F. Substrate solution
Weigh 20mg of cholesterol linoleate (produced by Nakarai Tesque Inc.) and dissolve in 4ml of 2-propanol with heating (Prepared solution). Mix 35ml of deionized water and 10ml of Triton X-100 solution (B). Keep the solution at 75 ± 2℃. Add Prepared solution slowly into the solution with stirring, then keep this mixture at 75 ± 2℃ for 30 minutes.
After cooling with running tap water, fill up the mixture to 50ml with deionized water. (Can be used 21 days if kept refrigerated)
G. Enzyme solution
Weigh some of Cholesterol Esterase “Amano” 2A and dissolve in 0.15 M Phosphate buffer (D).
Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.020 ± 0.005.

Procedure

Pipette 2.0ml of Aminoantipyrine-phenol solution (E) and 1.0ml of Substrate solution (F) respectively into a quartz cell (d=10mm), and keep the solution at 37 ± 0.5℃ for 10 minutes. Then, pipette 0.10ml of Enzyme solution (G) into the quartz cell and mix well immediately. Keep the reaction mixture at 37 ± 0.5℃. Exactly at 3 minutes and 6 minutes after the addition of Enzyme solution (G), measure the absorbances of the reaction mixture at 500nm (A3 and A6). As a blank, pipette 0.15M Phosphate buffer (D) into another quartz cell (d=10mm) instead of Enzyme solution (G) and take the same procedure described above (Ab3 and Ab6).

Calculation

$$\text{Cholesterol Esterase activity (u/mg)} = \frac{(A6-A3)-(Ab6-Ab3)}{3} \times \frac{1}{13.6} \times 2 \times 3.1 \times \frac{n}{0.1}$$

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

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