Cholesterol Oxidase“Amano”7

(Cholesterol: oxygen oxidoreductase, EC 1.1.3.6)

Cholesterol Oxidase“Amano”7 is a cholesterol oxidase preparation, manufactured using recombinant bacteria.

Catalysis

Cholesterol + O_2 ⟶ Cholest-4-en-3-one + H_2O_2

Specification and Preparation

Activity:	Cholesterol oxidase activity	≧ 30 u/mg
Appearance:	Yellow to orange powder, lyophilized	
Additive:	Trehalose	

Characteristics

1. Molecular weight: 50,000 (SDS-PAGE)
2. Km: 5.2×10^{-5} M
3. Optimum pH: 7.0
4. pH stability: 5.0-10.0 (40°C, 1hr)
5. Optimum temperature: 50°C
6. Thermal stability: up to 50°C (pH 7.0, 1hr)
7. Application: Used for the enzymatic determination of cholesterol in serum by coupling with cholesterol esterase 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 (nitrogen filled bottle).

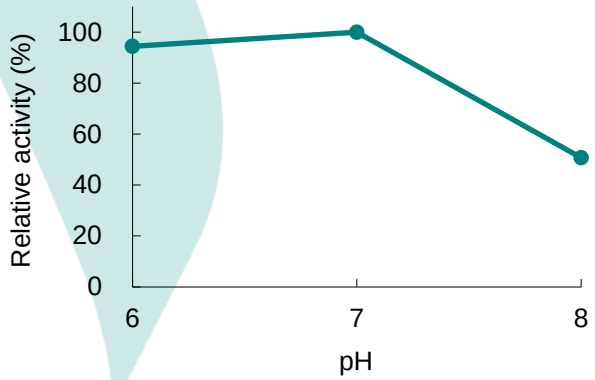
Safe Handling

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.

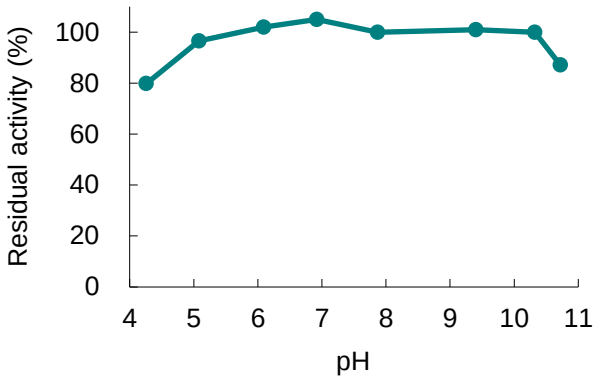
General properties

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

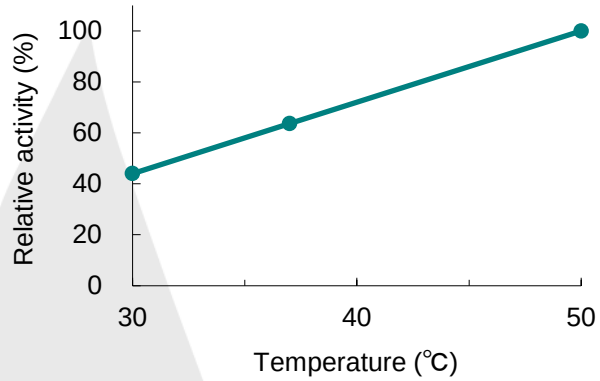
pH and Activity



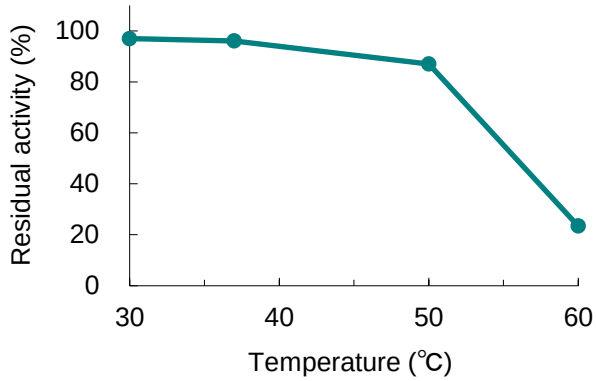
pH stability



Temperature and Activity



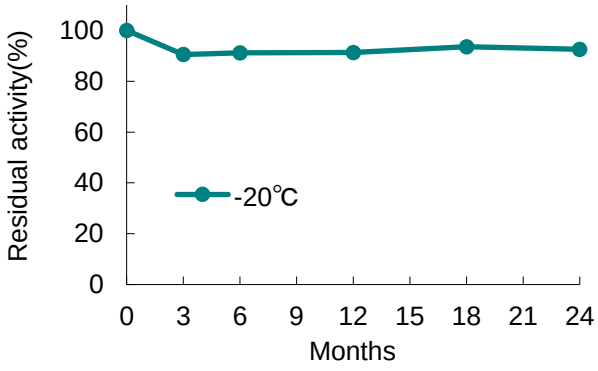
Thermostability



Effect of Various Chemicals

Chemical	Concentration	Residual Activity (%)
None	1mM	100
$MgCl_2$	1mM	100
$CaCl_2$	1mM	100
$FeCl_2$	1mM	92
$ZnCl_2$	1mM	97
$PbCl_2$	1mM	97
$CuCl_2$	1mM	92
Triton X-100	0.1%	100
Na-Cholate	0.1%	98
SDS	0.025%	95

Stability (powder form)



Assay method of Cholesterol Oxidase “Amano” 7

Principle

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

The appearance of Cholest-4-en-3-one is measured at 240 nm by spectrophotometry.

Unit Definition

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

Reagents

A. Triton X-100 solution (50 mg/ml deionized water)

B. Working solution (pH 7.0)
Weigh 5.28 g of KH_2PO_4 and 21.9 g of $Na_2HPO_4 \cdot 12H_2O$, then dissolve in 800 mL of deionized water. Add 10 mL of Triton X-100 solution (A), then adjust pH 7.0 with 4mol/L NaOH. Fill up to 1000 mL with deionized water. After preparation, release the lid of the container and allow the solution to stand under refrigeration for 24 hours before use. (Can be used for 3 months in refrigerated storage)

C. Substrate solution
Weigh 23.2 mg of cholesterol (FUJIFILM Wako Pure Chemical Corporation) and dissolve in 10 ml of 2-propanol. (Can be used for 5 days at room temperature)

D. Enzyme solution
Weigh some of Cholesterol Oxidase“Amano”7 and dissolve in chilled Working solution (B). Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.040±0.020. This reagent should be kept refrigerated and should be used within 2hr after enzyme dissolved.

Procedure

Pipette 3.0 ml of Working solution (B), 0.05 ml of Enzyme solution (D) respectively into a quartz cell (d =10 mm), and keep at 37±0.5℃ for 5 minutes. Then, pipette 0.05 ml of Substrate solution (C) into the quartz cell and mix well immediately. Keep the reaction mixture at 37±0.5℃. Exactly at 2 minutes and 4 minutes after the addition of Substrate solution (C), measure the absorbance of the reaction mixture at 240 nm (A2 and A4). As a blank, pipette Working Solution (B) into another quartz cell (d =10 mm) instead of Enzyme solution (D) and take the same procedure described above (Ab2 and Ab4).

Calculation

$$\text{Cholesterol Oxidase activity (u/mg)} = \frac{(A4-A2)-(Ab4-Ab2)}{2} \times \frac{1}{12.2} \times 3.1 \times \frac{n}{0.05}$$

2 Reaction time

12.2 Millimolar absorption coefficient of Cholest-4-en-3-one at 240 nm

3.1: Volume of the reaction mixture

0.05 Volume of Enzyme solution

n: Dilution factor of Enzyme solution

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