Cholesterol Dehydrogenase“Amano”6

(Cholesterol : NAD^+ oxidoreductase)

Cholesterol Dehydrogenase“Amano”6 is a cholesterol dehydrogenase preparation, manufactured using recombinant bacteria.

Catalysis

Cholesterol + NAD^+ ⇄ Cholest-4-en-3-one + NADH + H^+

Specification and Preparation

Activity:	Cholesterol dehydrogenase activity	≧ 18 u/mg (Amano method)
Contaminants:	NADH oxidase activity	≦ 5×10^{-2}%
	Glucose dehydrogenase activity	≦ 5×10^{-2}%
Appearance:	White to brown powder, lyophilized	
Additive:	Not added	

Characteristics

1. Molecular weight: 37,000 (SDS-PAGE)
2. Isoelectric point: 4.5
3. Km: 1.5×10^{-4} M (Cholesterol)
   2.3×10^{-4} M (NAD^+)
4. Optimum pH: 10.0
5. pH stability: pH 7.0 (37°C, 15 min)
6. Optimum temperature: 30°C
7. Thermal stability: up to 35°C (pH 7.0, 15 min)
8. Activator: Triton X-100
9. Inhibitor: Ag^+
10. Application: Used for the enzymatic determination of cholesterol in serum by coupling with cholesterol esterase in clinical diagnosis.
11. Note: Product may include 0.006-0.6% 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)

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

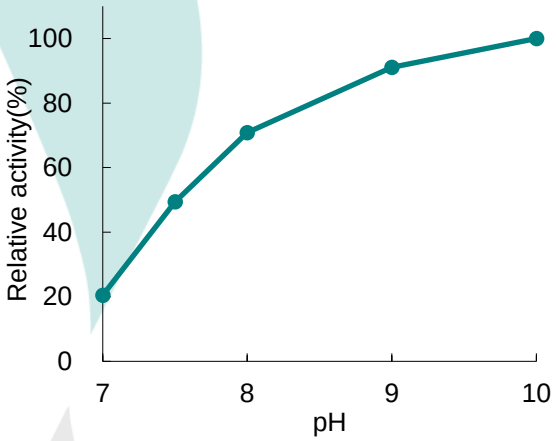
Safe Handling

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.

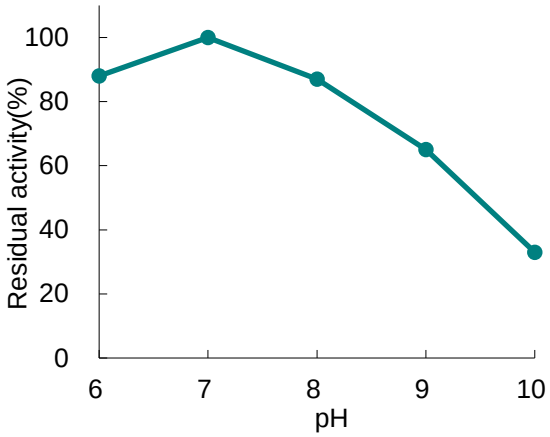
General properties

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

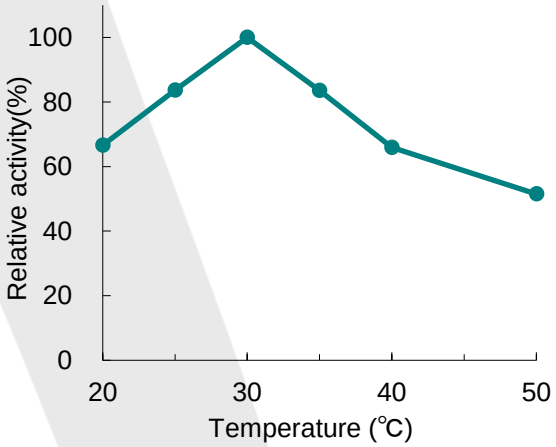
pH and Activity



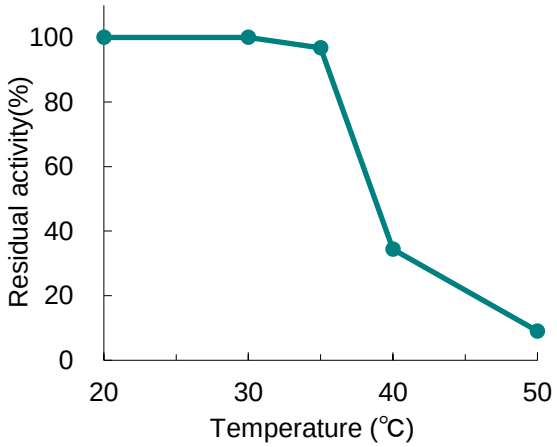
pH stability



Temperature and Activity



Thermostability



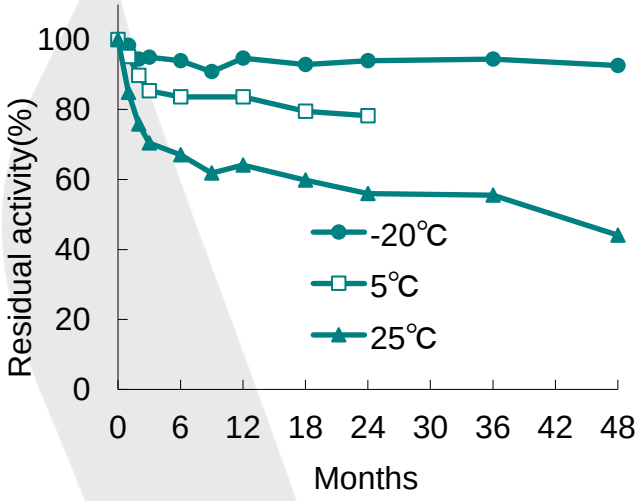
Substrate Specificity

Substrate	Relative Activity (%)
Cholesterol	100
β-Sitosterol	52
Ergosterol	50
Stigmasterol	30
Pregnenolone	14
Lanosterol	0
Dehydroisoandrosterone	0
Cholic acid	0
Testosterone	0
NAD	100
NADP	0

Effect of Various Chemicals

Chemical	Concentration (mM)	Relative Activity (%)
None		100
$MnCl_2$	1.0	99
$MgCl_2$	1.0	99
$CaCl_2$	1.0	93
$FeCl_2$	1.0	100
$LiCl_2$	1.0	96
$NiCl_2$	1.0	96
$ZnCl_2$	1.0	48
$AgNO_3$	1.0	0

Stability (powder form)



Assay method of Cholesterol dehydrogenase activity

Principle

$$\text{Cholesterol} + NAD^+ \xrightarrow{\text{Cholesterol Dehydrogenase}} \text{Cholest-4-en-3-one} + NADH + H^+$$

The appearance of NADH is measured at 340 nm by spectrophotometry.

Unit Definitio

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

Reagents

A. 50 mg/ml Triton X-100 solution (dissolved in deionized water)
B. 20 mg/ml Triton X-100 solution (dissolved in deionized water)
C. 0.3M Tris-HCl buffer (pH 8.5)
D. 0.02M Phosphate buffer (KH_2PO_4 – Na_2HPO_4, pH 7.0)
E. NAD solution
Weigh 75 mg of β-NAD^+ (Oriental Yeast Co., Ltd.) and dissolve in 0.3M Tris-HCl buffer (C). Fill up to 25 ml with 0.3M Tris-HCl buffer (C). (Can be used for 5 days if kept refrigerated)
F. Substrate solution
Transfer 40 ml of 20 mg/ml Triton X-100 solution (B) in a beaker (50 ml) and incubate at 75 – 80℃ with stirring. Weigh 50 mg of cholesterol (Nakarai Tesque Inc.) and add 4 ml of 2-propanol and dissolve at 75 – 80℃. Add the solution into 20 mg/ml Triton X-100 solution (B) in the beaker, then keep the mixture at 75 – 80℃ in a water bath for 30 minutes. After cooling with running tap water, fill up to 50 ml with 20 mg/ml Triton X-100 solution (B). (Can be used for 10 days if kept refrigerated)
G. Diluent
Mix 0.02M Phosphate buffer (D) and 5 ml of 50 mg/ml Triton X-100 solution (A), then fill up to 500 ml with 0.02M Phosphate buffer (D).
H. Enzyme solution
Weigh out Cholesterol Dehydrogenase“Amano”6 and dissolve in chilled Diluent (G).
Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.020±0.005.

Procedure

Pipette 2.0 ml of Substrate Solution (F) and 1.0 ml of NAD solution (E) respectively into a quartz cell (d =10 mm) and keep at 25±0.5℃ for 5 minutes. Then pipette 0.10 ml of Enzyme solution (H) into the quartz cell and mix well immediately. Keep the reaction mixture at 25±0.5℃. Exactly at 1 minute and 3 minutes after the addition of Enzyme solution (H), measure the absorbances of the reaction mixture at 340 nm (A1 and A3). As a blank, pipette Diluent (G) into another quartz cell (d =10 mm) instead of Enzyme solution (H) and take the same procedure described above (Ab1 and Ab3).

Calculation

$$\text{Cholesterol dehydrogenase activity (u/mg)} = \frac{(A3-A1)-(Ab3-Ab1)}{2} \times \frac{1}{6.22} \times 3.1 \times \frac{n}{0.1}$$

2: Reaction time
6.22: Millimolar absorption coefficient of NADH
3.1: Volume of the reaction mixture
0.1: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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