Lipoprotein Lipase“Amano”3

(Triacylglycerol acylhydrolase, EC 3.1.1.3)

Lipoprotein Lipase“Amano”3 is a lipoprotein lipase preparation manufactured by a unique fermentation process using a selected strain of bacteria.

Catalysis

Triglyceride + $3H_2O$ ⟶ Glycerol + 3RCOOH

Specification and Preparation

Activity:	Lipoprotein lipase activity	≧ 1,000 u/mg (Amano method)
Appearance:	White powder, lyophilized	
Additive:	Glycine	

Characteristics

1. Molecular weight: 33,000 (Gel filtration)
2. Isoelectric point: 4.3
3. Km: 3.95×10^{-3} M
4. Optimum pH: 7.0
5. pH stability: 5.0-9.0 (30°C, 24hrs)
6. Optimum temperature: 50°C
7. Thermal stability: up to 40°C (pH 7.0, 30 min)
8. Inhibitor: Sodium Cholate
9. Stabilizer: Ca^{2+}
10. Application: Used for the enzymatic determination of triglyceride in serum by coupling with the related enzymes 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.

The information and recommendations contained herein are to the best of our knowledge reliable according to the current scientific and technical level. However, depending upon use method and/or condition, nothing herein is to be construed as a warranty or representation in respect otherwise, including freedom from patent infringement. Users shall make their own test and investigation for their particular purpose. We do not accept any liability for any loss, damage or infringement arising from the use of information and recommendations contained herein.

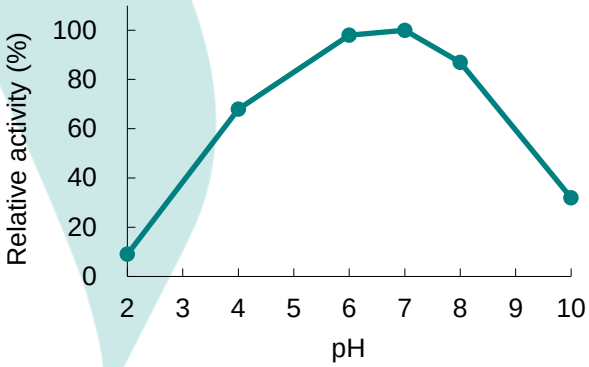
Safe Handling

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.

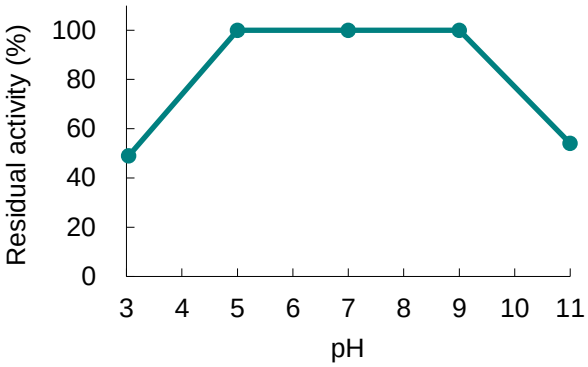
General properties

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

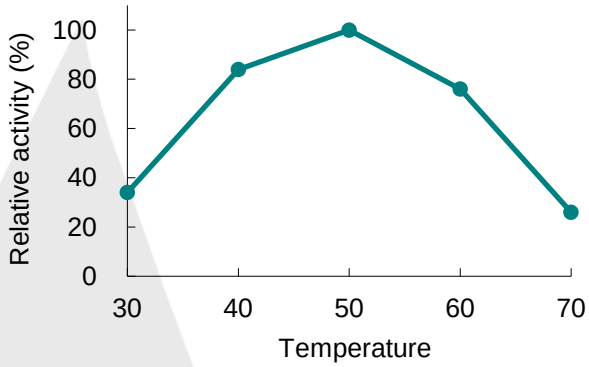
pH and Activity



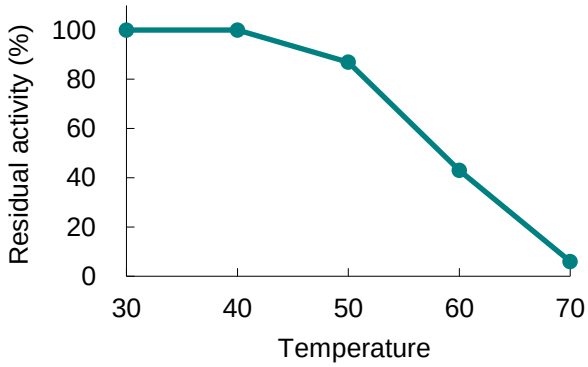
pH stability



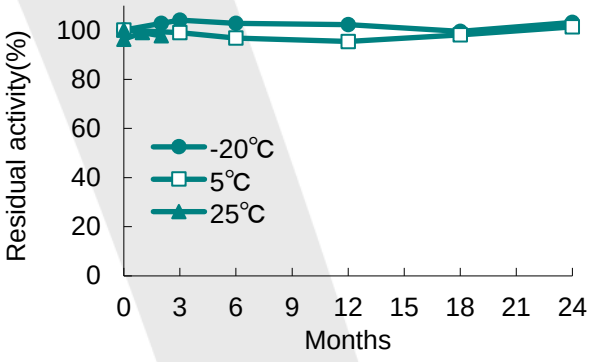
Temperature and Activity



Thermostability



Stability (powder form)



Assay method of Lipoprotein lipase activity

Principle

$$\text{Triglyceride} + 3H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + 3\ \text{Fatty acid}$$

$$\text{Fatty acid} + KOH \xrightarrow{\text{(Titration)}} K\text{-Fatty acid} + H_2O$$

The assay is based on the titration of fatty acid liberated from the above reaction.

Unit Definition

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

Reagents

A. 2/15 M Phosphate buffer (KH_2PO_4-Na_2HPO_4, pH7.0)

B. 1/15 M Phosphate buffer (KH_2PO_4-Na_2HPO_4, pH 7.0)

C. Substrate solution
Weigh 1.60 g of bovine serum albumin (FUJIFILM Wako Pure Chemical Corporation: Fraction V) and dissolve in 16 ml of 2/15 M Phosphate buffer (A). Add 24 ml of 10% Intralipos (Otsuka Pharmaceutical Co., Ltd.: soybean oil) into the solution and stir well. (Can be used only on the day of preparation if refrigerated)

D. Heptane -2-propanol-sulfuric acid solution
Mix 75ml of heptane, 300 ml of 2-propanol, and 7.5 ml of 1 mol/L sulfuric acid successively. (Can be used for 3 months at room temperature)

E. Cresol red indicator
Weigh 50 mg of cresol red and dissolve in 50 ml of ethanol. Filtrate with filter paper. (Can be used for 1 month if stored in a brown bottle at room temperature or in a refrigerator)

F. Triton X-100 stock solution (5g/100ml deionized water)

G. Diluent
Mix approx. 900ml of 1/15 M Phosphate buffer (B) and 2 ml of Triton X-100 stock solution (F), then fill up to 1000 ml with 1/15 M Phosphate buffer (B).

H. Enzyme solution
Weigh out Lipoprotein Lipase“Amano”3 and gently dissolve in chilled Diluent (G).
Enzyme solution should be prepared so that the value of (T20-T0) becomes in the range of 0.25 – 0.50 ml.
Do not shake the Enzyme solution vigorously, or the enzyme becomes inactive.

I. 0.01N KOH-ethanol solution
Pipette 5 ml of 0.1 mol/L potassium hydroxide ethanolic solution (Commercial product, for volumetric analysis) into a volumetric flask (50 ml) and fill up to 50 ml with ethanol.

Procedure

Pipette 1.0 ml of 2/15M Phosphate buffer (A) and 2.0 ml of Substrate solution (C) respectively into a test tube (Φ25 x 200 mm) with a ground stopper and mix well. Stand the solution in a water bath shaker at 37 ± 0.5℃ for more than 10 minutes, then add 1.0 ml of Enzyme solution (H) and incubate with shaking.
Exactly 20 minutes after addition of Enzyme solution (H), add 10.0 ml of Heptane-2-propanpol-sulfuric acid solution (D), then shake and mix well. Add 6.0 ml of heptane and 4.0 ml of deionized water, then cap the stopper and shake using shaker for 5 minutes.
After shaking, stand the reaction mixture in tap water for more than 20 minutes to make heptane layer separate from water layer. Pipette 6.0 ml of upper layer (heptane layer) into a test tube (Φ15 x 105 mm) and add 2 drops of Cresol red indicator (E). Then, titrate the solution with 0.01N KOH-ethanol solution (I) in nitrogen currents while stirring (T20 ml). It is the end point that the color of the solution turns violet.
As a blank, pipette 1.0 ml of 2/15 M Phosphate buffer (A) and 2.0 ml of Substrate solution (C) into another test tube (Φ25 x 200 mm) with ground stopper, then add 10.0 ml of Heptane-2-propanol-sulfic acid solution (D) and mix well. Add 1 ml of Enzyme solution (H) and take the same procedure described above (T0 ml)

Calculation

$$\text{Lipoprotein lipase activity (u/mg)} = \frac{(T20 - T0)}{20} \times 10 \times 1.327 \times f \times n$$

20: Reaction time
10: Conversion factor (1 ml of 0.01N KOH-ethanol solution corresponds to 10 μ moles of fatty acid)
1.327: Total heptane / 6 ml of heptane = (6+10/51×10) / 6 = 1.327
f: Normality coefficient of 0.01N KOH-ethanol 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	