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脂肪酶 PS-IM

ORIGIN： Burkholderia sp. (formerly Pseudomonas sp.)

CATALYSIS：

Triglyceride + $3H_2O$ → Glycerol + 3RCOOH

SPECIFICATIONS：

Activity: LPL - 3 ≥ 1,000 u/mg

Appearance: White powder, lyophilized

Contaminants: Oxidase free

Stability: Stable for 24 months at -20°C

Additives: Glycine

CHARACTERISTICS：

Molecular weight: 33,000 (Gel filtration)

Isoelectric point: 4.3

Km value: 3.95×10^{-3} M

Inhibitor: Sodium cholate

Stabilizer: Ca^{2+}

Opt. pH: 7.0 - Fig. 1

Opt. temperature: 50°C - Fig. 2

Stable pH range: 5.0 – 10.0 (30°C, 24 hrs.) - Fig. 3

Stable temp. range: below 45°C (pH7.0, 30 min.) - Fig. 4

APPLICATIONS:

This enzyme is used for the enzymatic determination of triglyceride in serum by coupling with the related enzymes in clinical diagnosis.

PRESERVATION:

Below -20°C in a dry place- Fig. 5

Fig. 1 pH-Activity

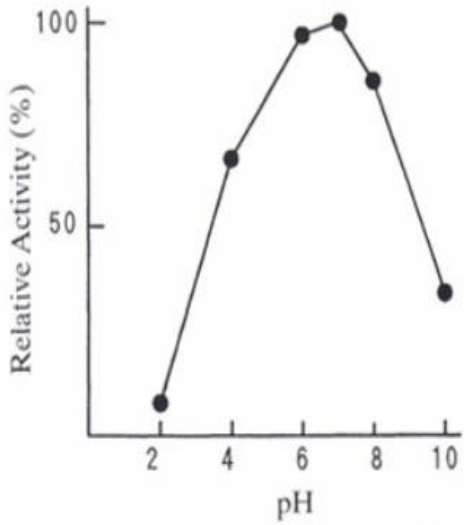


Fig. 2 Thermoactivity

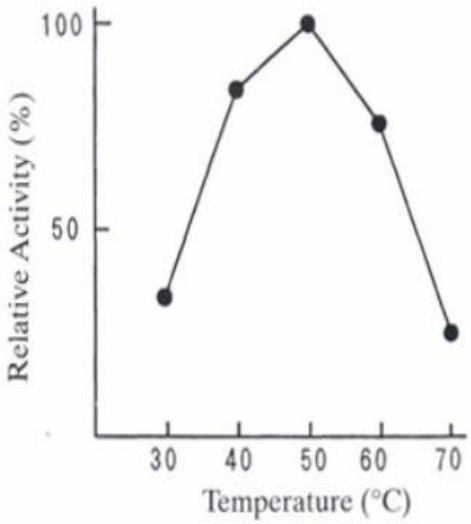


Fig. 3 pH-Stability

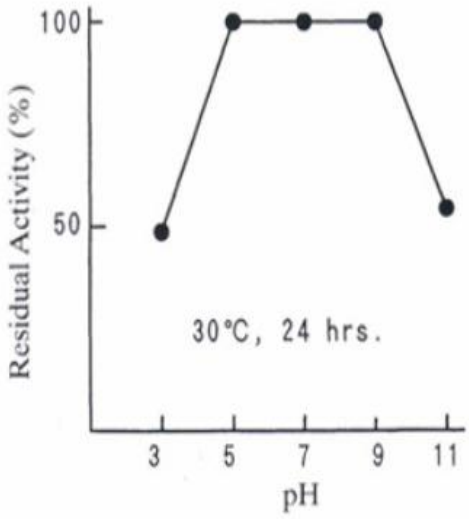


Fig. 4 Thermostability

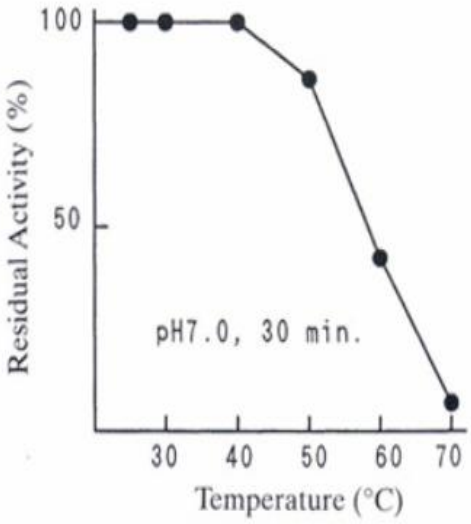
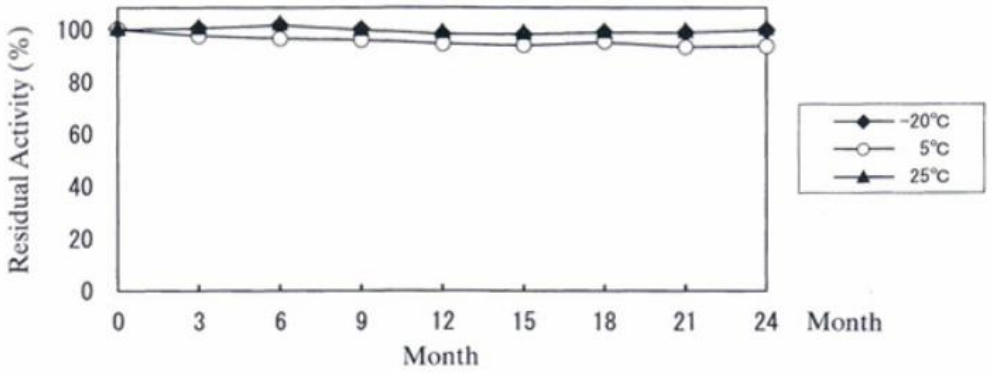


Fig. 5 Stability (Powder Form)



Principle：

Triglyceride + $3H_2O$ $\xrightarrow{\text{(Lipoprotein Lipase)}}$ Glycerol + 3 Fatty acid

Fatty acid + KOH $\xrightarrow{\text{(Titration)}}$ K - 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 produces one μ mole of fatty acid per minute under the conditions described below.

Reagents：

A. 1/7.5M Phosphate Buffer (KH_2PO_4,Na_2PO_4, pH 7.0)

B. Substrate Solution: Weigh 1.60 g of bovine serum albumin and dissolve in 16 ml of 1/7.5M Phosphate Buffer (A). Add 24 ml of Intralipids (produced by Wilfide Co.: soybean oil) into the solution and stir well. (Make a fresh solution for each use.)

C. Heptane - 2 - propanol - Sulfuric Acid Solution: Mix 10 parts of n - heptane, 40 parts of 2 - propanol, and 1 part of 2 N sulfuric acid successively.

D. Cresol Red Indicator: Weigh 50 mg of cresol red and dissolve in 50 ml of ethanol. Filtrate with filter paper.

E. 1/15 M Phosphate Buffer (containing 100 ppm of Triton X - 100, pH 7.0)

F. Enzyme Solution: Weigh approx. 100 mg of Lipoprotein Lipase “Amano” 3 and dissolve in chilled 1/15M Phosphate Buffer (E). Stand the solution until the enzyme is dissolved, then transfer the solution to a volumetric flask. (Shake the solution gently, when the enzyme sample is not dissolved.)

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, the enzyme becomes inactive.

G. 0.01N KOH - Ethanol Solution: Pipette 1 ml of 0.5 mol potassium hydroxide ethanol solution into a volumetric flask (50 ml), and fill up to 50 ml with ethanol.

Procedure：

1.Pipette 1.0 ml of 1/7.5M Phosphate Buffer (A) and 2.0 ml of Substrate Solution (B) respectively into a test tube (φ25×200 mm) with a ground stopper, and mix well. Stand the solution in a shaking incubator at 37 ± 0.1°C for more than 10 minutes, then add 1.0 ml of Enzyme Solution (F) and incubate with shaking for 20 minutes.

2.Just 20 minutes after the addition of Enzyme Solution (F), add 10.0 ml of Heptane - 2 - propanol - Sulfuric Acid Solution (C) and shake well. Add 6.0 ml of n - heptane and 4.0 ml of deionized water, then shake the solution.

3.After shaking, stand the reaction mixture in tap water for more than 20 minutes in order to make heptane layer separate from water layer.

Calculation：

Lipoprotein Lipase activity (u/mg) = [(T20 - T0) / 20] × 10 × 1.327 × f × Dm