(Diagnostic Reagent Grade) T-01

LIPASE [LP]

from *Chromobacterium viscosum*
(Triacylglycerol acylhydrolase, EC 3.1.1.3)
(Triacylglycerol lipase)

Triglyceride + 3 H_2O ⟶ Glycerol + 3 Fatty Acid

Preparation and Specification

Appearance : White to off-white amorphous powder, lyophilized
Specific activity : More than 2,500 U/mg solid
Contaminants :
Cholesterol oxidase Less than 0.01 % (U/U)
Catalase Less than 0.01 % (U/U)

L

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 120 kDa (pH 3.7) (gel filtration) 30 kDa (pH 7.3) (gel filtration)	
Isoelectric point	: pH 3.7 and pH 7.3	
Optimum pH	: 3.0–10.0	Figure 1
pH stability	: 4.0–10.0 (50℃, 60 min)	Figure 2
Thermal stability	: Stable at 70℃ and below (pH 7.0, 10 hr)	Figure 3
Storage stability	: At least one year at –20℃	Figure 4
Effect of various chemicals	: See Table 2	
Activators	: Detergents	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **triglyceride** when coupled with glycerophosphate oxidase (T–60) and glycerol kinase (T–64).

$$\text{TG} + 3\,H_2O \xrightarrow{\text{LP}} \text{Glycerol} + 3\,\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GKZ}} \text{G-3-P} + \text{ADP}$$

$$\text{G-3-P} + O_2 \xrightarrow{\text{GPOSP}} \text{DHAP} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

TG: Triglyceride
FFA: Free fatty acid
DHAP: Dihydroxyacetone phosphate

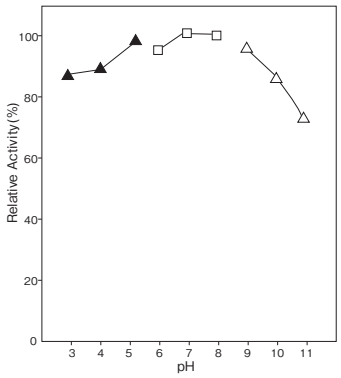
Table 1. Substrate specificity

Substrate	Relative activity (%)
Triolein	100
Tripalmitin	22
Trimyristin	53
Trilaurin	103
Tricaprin	166
Tricaprylin	312
Tricaproin	156
Tributyrin	94
Tripropionin	22
Triacetin	38

Table 2. Effect of various chemicals on LP activity

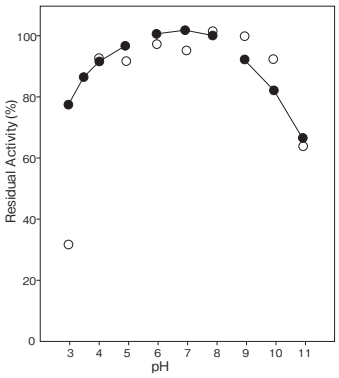
Additives	Concentration	Relative activity (%)
None	-	100
NiCl	1mM	97
$MnCl_2$	1mM	98
$(NH_4)_2SO_4$	1mM	100
$MgCl_2$	1mM	99
ZnCl	1mM	94
$ZnSO_4$	1mM	94
$Ba(CH_3COO)_2$	1mM	98
$CaCl_2$	1mM	100
$MoSO_4$	1mM	106
$CuSO_4$	0.5mM	26
$CuCl_2$	0.5mM	26
$FeCl_3$	1mM	103
$CoCl_2$	1mM	96
Li_2CO_3	1mM	93
EDTA	1mM	105
KCl	100mM	100
NaCl	100mM	98
NaN_3	0.05%	97
NaF	20mM	88

Fig.1 pH Optimum



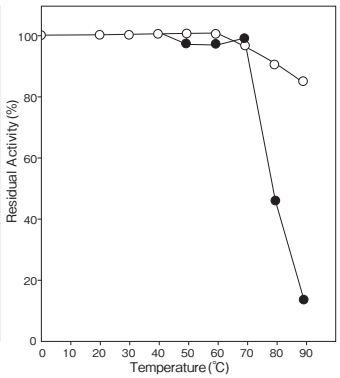
▲ : McIlvaine buffer
□ : Phosphate buffer
△ : Borate buffer

Fig.2 pH Stability



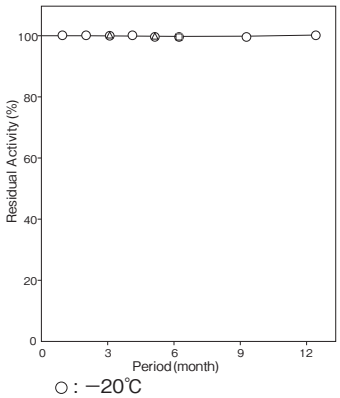
● : 37℃, 24 hr
○ : 50℃, 1 hr
pH 3, 4, 5 McIlvaine buffer
pH 6, 7, 8 Phosphate buffer
pH 9, 10, 11 Borate buffer

Fig.3 Thermal Stability



pH 7.0, 10 hr
○ : Lyophilized powder
● : Aqueous solution

Fig.4 Storage (lyophilized powder)



○ : −20℃
□ : 5℃
△ : 30℃

Assay

■ Principle

The assay is based on the titration of fatty acids liberated in the following reactions:

$$\text{Triglyceride} + 3\,H_2O \xrightarrow{\text{LP}} \text{Glycerol} + 3\,\text{Fatty acid}$$

(Titration)

$$\text{Fatty acid} + \text{NaOH} \longrightarrow \text{Na-Fatty acid} + H_2O$$

■ Unit definition

One unit is defined as the amount of enzyme which liberates 1 μ mole of fatty acid per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Substrate suspension (Olive oil and Adekatol SO-120)
   50 g of Olive oil (Japanese Pharmacopoeia grade) and 50 g of Adekatol SO-120 are suspended with 150 ml of distilled water.
2. Reaction stopper
   Mixture of ethanol and acetone (1:1)
3. Indicator
   1% (W/V) Phenolphthalein-ethanol solution
4. Titration solution
   50 mM NaOH solution
5. Enzyme dilution buffer
   0.1 M KH_2PO_4-NaOH buffer, pH 8.0 containing 0.1% (W/V) BSA and 0.1% (W/V) NaN_3
6. Reagents
   Olive oil: (Japanese Pharmacopoeia grade)
   Ethanol: (Japanese Pharmacopoeia grade)
   Adekatol SO-120 : ADEKA CORPORATION
   BSA: Millipore Fraction V pH5.2 #81-053

■ Enzyme solution

Accurately weigh about 10 mg of the sample and add enzyme dilution buffer to make a total of 50 ml.
Dilute it with enzyme dilution buffer to adjust the concentration to within 2-4 U/ml.

■ Procedure

1. Pipette accurately 5 ml of substrate suspension and 2 ml of distilled water into a test tube (24 mm i.d. × 200 mmH) and mix to start the preincubation at 37℃.
2. After 10 min, add 0.5 ml of enzyme solution and mix to start the reaction.
   ※ In the case of a test blank, add 0.5 ml of enzyme dilution buffer in place of enzyme solution.
3. After 20 min, stop the reaction with 16 ml of reaction stopper.
4. Add 3 drops of indicator and titrate the whole mixture with under nitrogen gas bubbling.
   ※ End point of titration: Appearance of the same color as that of the blank

   Titration volume sample : Vs
   blank : Vc

   $\triangle V = (Vs - Vc) \leqq 2.5$ ml

   $Vc \leqq 0.6$ ml

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle V \times F}{20} \times 50 \times \frac{1}{0.5} \times \frac{1}{X}$$

20 : reaction time (min)
F : factor of titration solution (50 mM NaOH)
50 : concentration (mM) of titration solution (50 mM NaOH)
0.5 : the volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 4).

References

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LP 活性測定法（Japanese）

Ⅰ．試薬液

1. 基質懸濁液（オリーブ油とアデカトール SO-120 の懸濁液）
   「局方」オリーブ油 50.0g とアデカトール SO-120 50.0g を精製水 150ml に懸濁する。
2. 反応停止液
   エタノール－アセトン（1:1）混液
3. 指示液
   1%（W/V）フェノールフタレン－エタノール溶液
4. 滴定液
   50mM NaOH 液
5. 酵素溶解希釈用液
   0.1%(W/V)BSA と 0.1%(W/V)NaN_3 を含む 0.1M KH_2PO_4-NaOH 緩衝液 pH8.0
6. 試薬
   オリーブ油:「局方」
   エタノール:「局方」
   アデカトール SO-120:ADEKA 製
   BSA: Millipore 製　Fraction V pH5.2 #81-053

Ⅱ．酵素試料液

検品約 10mg を精密に量り、酵素溶解希釈用液に溶解して全容 50ml とする。
その液を酵素溶解希釈用液で 2～4U/ml 濃度となるように適宜希釈する。

Ⅲ．測定操作法

1. 試験管（24mm i.d. × 200mm H）に基質懸濁液 5ml と精製水 2ml を正確に分注して攪拌混和後、37℃で予備加温する。
2. 10 分経過後、酵素試料液 0.5ml を加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 0.5ml を加える。
3. 20 分経過後、反応停止液 16ml を加えて反応を停止する。
4. 指示液 3 滴を加えて N_2 ガスで攪拌しながら滴定液で滴定する。
   ※滴定の終点は盲検時と同色を呈した時点とする。
   求められた滴定量を試料液は Vs、盲検液は Vc とする。

   $\Delta V = (Vs - Vc) \leqq 2.5$ ml

   $Vc \leqq 0.6$ ml

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta V \times F}{20} \times 50 \times \frac{1}{0.5} \times \frac{1}{X}$$

20 : 反応時間（min）
F : 滴定液（50mM NaOH）の Factor
50 : 滴定液（50mM NaOH）の濃度（mM）
0.5 : 反応に供した酵素試料液量（ml）
X : 酵素試料液中の検品濃度（mg/ml）