LIPASE [LPBP]

from Microorganism
(Triacylglycerol acylhydrolase, EC 3.1.1.3)
(Triacylglycerol lipase)

Triglyceride + $3H_2O$ ⟶ Glycerol + 3 Fatty Acid

★ Advantages
① Low adsorption onto cuvette
② Stability in solution

Preparation and Specification

Appearance : White to light brownish amorphous powder, lyophilized
Specific activity : More than 800 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 55 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.9 ± 0.2	
Optimum pH	: 4.2	Figure 1
pH stability	: pH 3.5-7.0 (45℃, 60 min)	Figure 2
Optimum temperature	: 37℃ (Phosphate buffer)	Figure 3
Thermal stability	: Stable at 37℃ and below (pH 7.5, 30 min)	Figure 4
Effect of metal ions	: See Table 2	
Low adsorption	: See Figure 5	
Liquid stability	: See Figure 6	
High reactivity after long storage	: See Figure 7	
Effect of various chemicals	: See Table 3	
Effect of detergents	: See Table 4	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **triglyceride**.

$$\text{TG} + 3\,H_2O \xrightarrow{\text{LPBP}} \text{Glycerol} + 3\ \text{Fatty acid}$$

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

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

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

TG: Triglyceride DHAP: Dihydroxyacetonephosphate

Table 1. Substrate specificity

Substrate (13.3%)	Relative activity (%)
Triolein	100
Trimyristin	14.8
Trilaurin	34.9
Tricaprylin	92.2
Tricaprin	87.1
Tricaproin	13.5
Tributyrin	38.0
Triacetin	0

Table 2. Effect of metal ions on LPBP activity

Metal ion (1mM)	Relative activity (%)
None	100
NaCl	100
KCl	95.8
LiCl	95.8
$MgCl_2$	97.9
$CaCl_2$	101
$CuCl_2$	106
$MnCl_2$	103
$ZnCl_2$	104
$FeCl_2$	95.8
$CoCl_2$	100
$NiCl_2$	104
$BaCl_2$	99.0

Table 3. Effect of various chemicals on LPBP activity

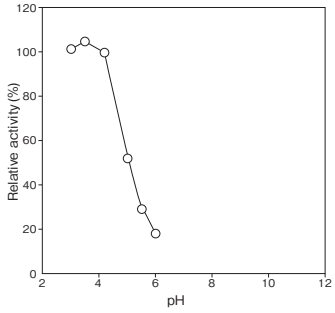
Chemical (1mM)	Relative activity (%)
None	100
NaN_3	99.0
NaF	60.4
EDTA	106

Table 4. Effect of detergents on LPBP activity

Detergent (0.1%)	Relative activity (%)
None	100
Adekanol NP695	91.9
Adekanol NP720	96.2
Adekanol SO120	86.7
Adekanol B795	88.1
Triton X305	91.9
Emulgen B66	87.9

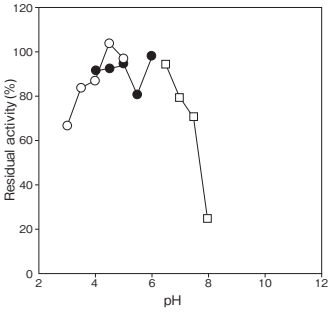
L

Fig.1 pH Optimum



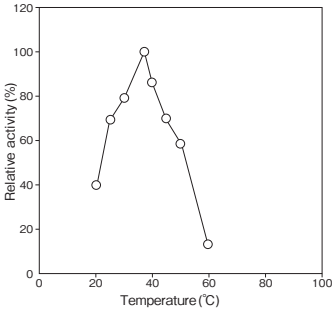
○ : MacIlvaine buffer

Fig.2 pH Stability



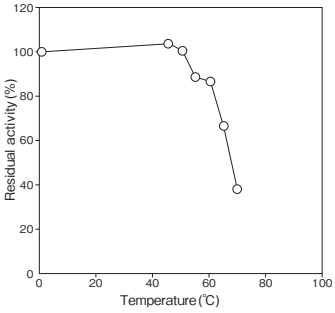
45℃, 60 min.
○ : Citrate buffer
● : 3,3-Dimethylglutarate-NaOH buffer
□ : Phosphate buffer

Fig.3 Optimum Temperature



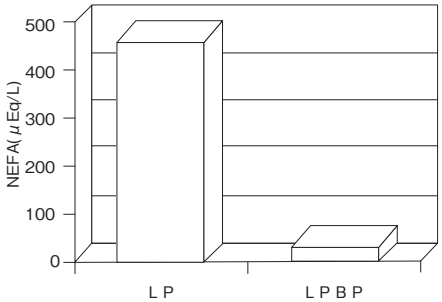
pH 6.8
40 mM Phosphate buffer

Fig.4 Thermal Stability



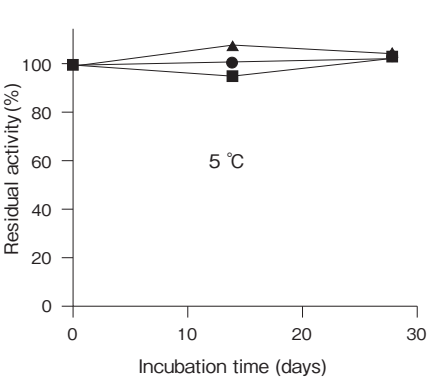
pH 7.5, 30 min.
40 mM Phosphate buffer

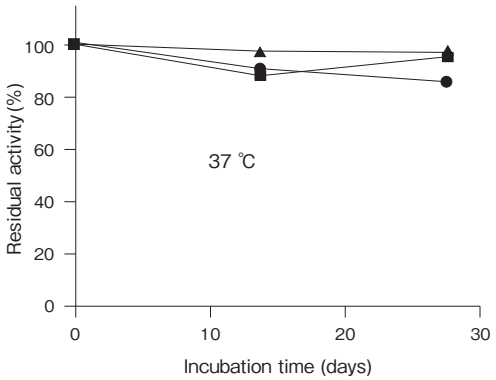
Fig.5 Influence on the measurement of NEFA after TG assay demonstrating low LPBP adsorption



Autoanalyzer : HITACHI 7150

Fig.6 Stability of reconstituted LPBP

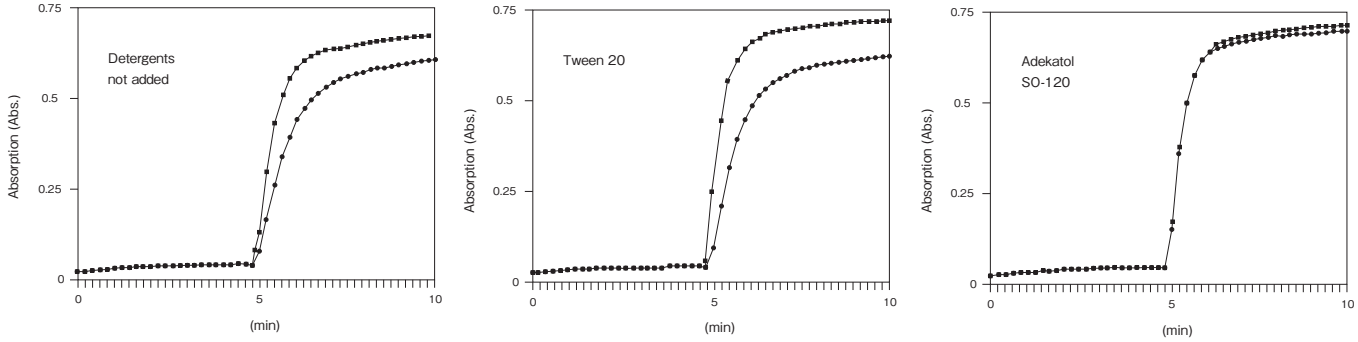




■ : detergent not added
● : Adekanol B-795
▲ : Adekatol SO-120

Storage conditions : 20mM Good's buffer, pH 5.5
0.06% 4-amino antipyrine
0.05% NaN_3, 0.05% detergent

Fig.7 Reactivity of LPBP after long-term storage in liquid form



■ : initial
● : at 25℃,4 weeks later

Storage conditions: 250U/ml LPBP, 0.25 U/ml MGLP
0.05% Tween 20 or Adekatol SO-120

Assay

■ Principle

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

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

$$\text{Fatty acid} + NaOH \xrightarrow{\text{(Titration)}} \text{Na–Fatty acid} + H_2O$$

■ Unit definition

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

■ Reagents

1. McIlvaine buffer pH 4.2
   Mix 0.2 M Na_2HPO_4 and 0.1 M citrate solution and adjust pH to 4.2 at 25℃.
2. Substrate suspension (Olive oil and Adekatol SO–120)
   50g of Olive oil (Japanese Pharmacopoeia grade) and 50g of Adekatol SO–120 are suspended with 150 ml of distilled water.
3. Reaction stopper
   Mixture of ethanol and acetone (1:1)
4. Indicator
   1% (W/V) Phenolphthalein–ethanol solution
5. Titration solution
   50 mM NaOH solution
6. Enzyme dilution buffer
   0.1 M KH_2PO_4–NaOH buffer, pH 6.0 containing 0.1% (W/V) BSA and 0.1% (W/V) NaN_3
7. 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 10 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 5 ml of substrate suspension and 2 ml of McIlvaine buffer into test tube (24 mm i.d. × 200 mmH) and mix to preincubate at 37℃.
2. After 10 min, add 0.50 ml of enzyme solution and mix to start the reaction.
   ※ In the case of a test blank, add 0.50 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 under nitrogen gas bubbling.
   ※ End point of titration: Appearance of the same color as that of the blank

   Titration volume sample : Vs
   blank : Vc

   $\triangle V = (Vs - Vc) \leqq 1.5$ 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.

References

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LPBP 活性測定法（Japanese）

Ⅰ．試薬液

1. McIlvaine 緩衝液 pH4.2
   0.2M Na_2HPO_4 溶液と 0.1M クエン酸溶液を混合して pH4.2（25℃）に調整する。
2. 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。
3. 反応停止液
   エタノール－アセトン（1:1）混液
4. 指示液
   1%（W/V）フェノールフタレン－エタノール溶液
5. 滴定液
   50mM NaOH 液
6. 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 pH6.0
7. 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、酵素溶解希釈用液で溶解して全容 10ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 試験管（24mm i.d × 200mm H）に基質懸濁液 5.0ml と McIlvaine 緩衝液 2.0ml 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 0.50ml 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 0.50ml を加える。
3. 20 分経過後、反応停止液 16.0ml 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（淡赤色）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。

$$\Delta V = (Vs - Vc) \leqq 1.5\ ml$$

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）