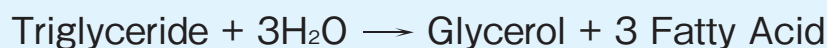


LIPASE [LPBP]

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



- ★ Advantages
- ① Low adsorption onto cuvette
 - ② Low liquid stability

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°C, 60 min) Figure 2
Optimum temperature : 37°C (Phosphate buffer) Figure 3
Thermal stability : Stable at 37°C 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**.

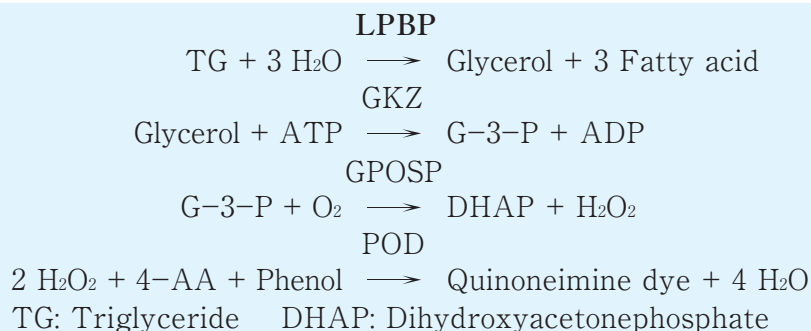


Table 1. Substrate specificity

Substrate (13.3%)	Relative activity (%)
Triolein	100
Trimyristin	14.8
Trilaurin	34.9
Tricaprylin	92.2
Tricaprin	87.1
Tricaproin	13.5
Tributylin	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

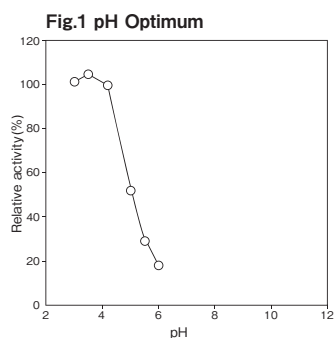


Fig.1 pH Optimum

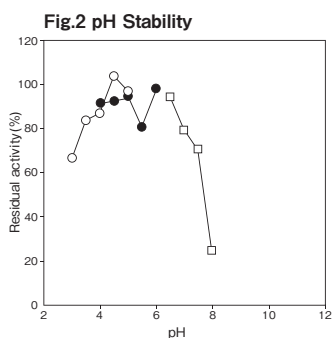


Fig.2 pH Stability

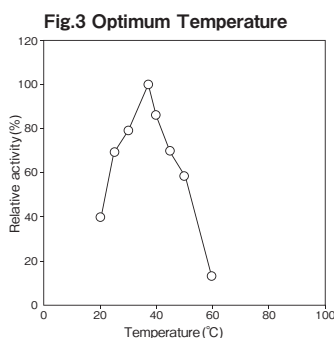


Fig.3 Optimum Temperature

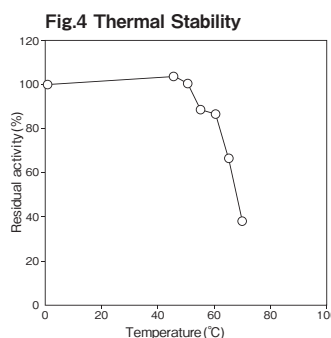


Fig.4 Thermal Stability

○ : MacIlvaine buffer

○ : Citrate buffer
● : 3,3-Dimethylglutarate-NaOH buffer
□ : Phosphate buffer

pH 6.8
40 mM Phosphate buffer

pH 7.5, 30 min.
40 mM Phosphate buffer

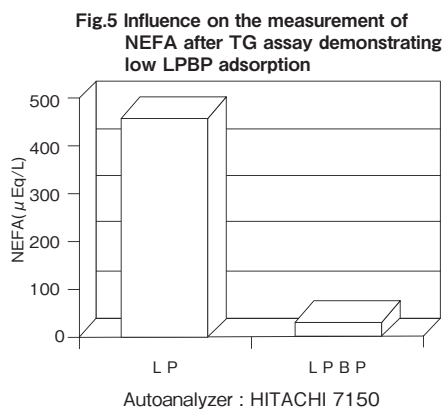


Fig.5 Influence on the measurement of NEFA after TG assay demonstrating low LPBP adsorption

Autoanalyzer : HITACHI 7150

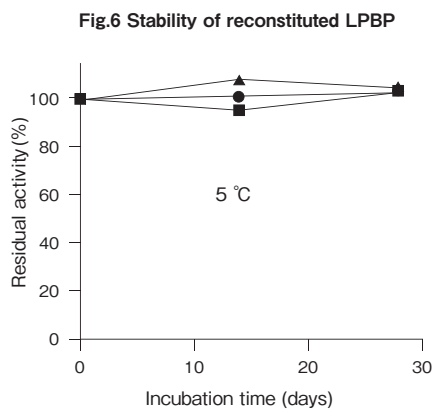
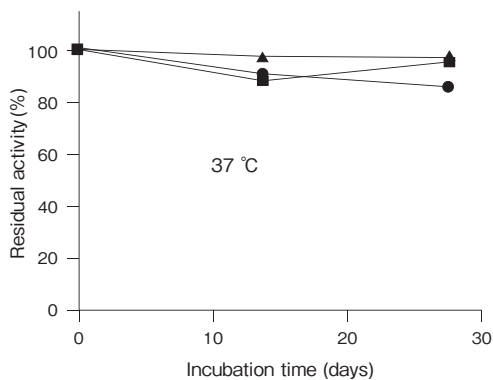


Fig.6 Stability of reconstituted LPBP

5 °C

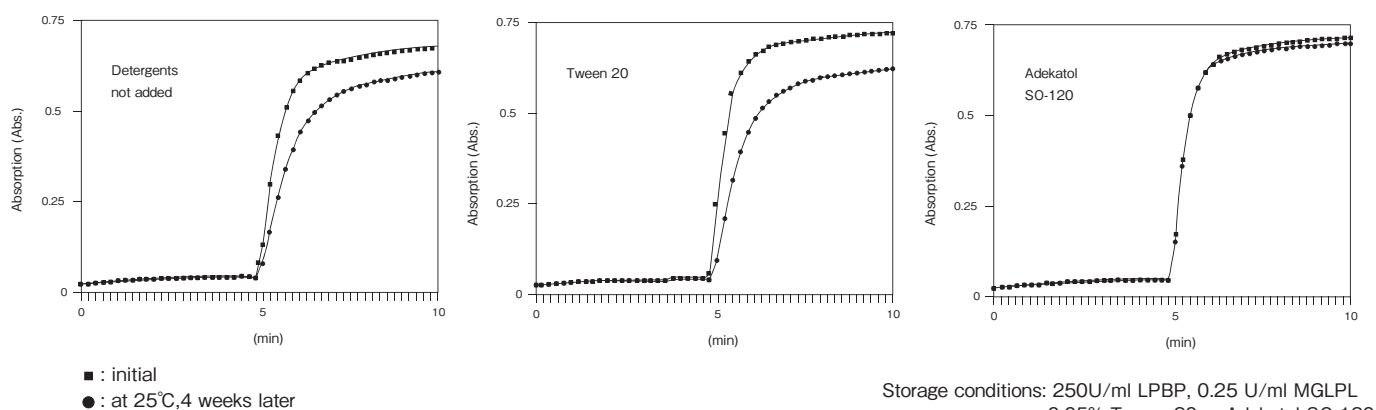
■ : detergent not added
● : Adekanol B-795
▲ : Adekatol SO-120



37 °C

Storage conditions : 20mM Good's buffer, pH 5.5
0.06% 4-amino antipyrine
0.05% NaN₃, 0.05% detergent

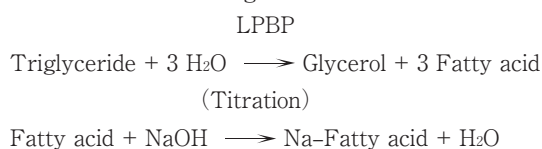
Fig.7 Reactivity of LPBP after long-term storage in liquid form



Assay

Principle

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



Unit definition

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

Reagents

- 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°C.
- Substrate suspension (Olive oil and Adekatal SO-120)
50g of olive oil (medical use) and 50g of Adekatal SO-120 are suspended with 150 ml of distilled water.
- Reaction stopper
Mixture of ethanol and acetone (1:1)
- Indicator
1% (W/V) Phenolphthalein-ethanol solution
- Titration solution
50 mM NaOH solution
- 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
- Reagents
Olive oil: (Japanese Pharmacopoeia grade)
Ethanol: (Japanese Pharmacopoeia grade)
Adekatal 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

- Pipette accurately 5 ml of substrate suspension and 2 ml of McIlvaine buffer into test tube (24 mm i.d. \times 200 mmH) and mix to preincubate at 37°C.
- 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.
- After 20 min, stop the reaction with 16 ml of reaction stopper.
- 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

$$\begin{array}{l} \text{Titration volume} \quad \text{sample} \quad : V_s \\ \quad \quad \quad \quad \quad \quad \text{blank} \quad : V_c \end{array}$$

$$\Delta V = (V_s - V_c) \leq 1.5 \text{ ml}$$

Calculation

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

- 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°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

References

1. Yamaguchi, T., Muroya, N., Isobe, M. and Sugiura, M. (1973) Agric. Biol. Chem., **37**, 999-1005.
2. Sugiura, M., Isobe, M., Muroya, N. and Yamaguchi, T. (1974) Agric. Biol. Chem., **38**, 947-952.
3. Sugiura, M. and Isobe, M. (1974) Biochem. Biophys. Acta, **341**, 195-200.
4. Sugiura, M. and Isobe, M. (1975) Chem. Pharm. Bull., **23**, 1226-1230.
5. Horiuchi, Y., Koga, H. and Gocho, S. (1976) J. Biochem., **80**, 367-370.
6. Saiki, T., Takagi, Y., Suzuki, T., Narasaki, T., Tamura, G. and Arima, K. (1969) Agric. Biol. Chem., **33**, 414.

LPBP 活性測定法 (Japanese)

I. 試薬液

1. McIlvaine 緩衝液 pH4.2
0.2M Na₂HPO₄ 溶液と 0.1M クエン酸溶液を混合して pH4.2 (25°C) 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) NaN₃ を含む
0.1M KH₂PO₄-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 社製 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°C 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°C 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 N₂ 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 V_s、盲検液は V_c とする。

$$\Delta V = (V_s - V_c) \leq 1.5 \text{ ml}$$

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)