

LIPASE [LPM]

mixture of LP(T-01) and MGLP II(T-117)



- ★ Advantages
- ① High Reactivity
 - ② Thermal stability
 - ③ Stability in solution

Preparation and Specification

Appearance : White to pale brownish amorphous powder, lyophilized
 Specific activity : LP ; 1500-3500 U/mg
 MGLP II ; 12-30 U/mg

Applications for Diagnostic Test

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

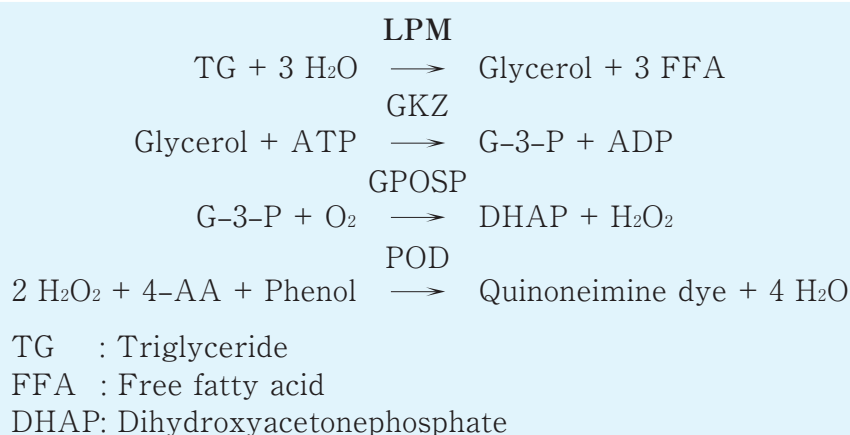


Fig.1 Thermal stability

Stored in R2 of TG reagent
Residual activity (%) after 30 min. at each temperature
Activity assay method: 37°C

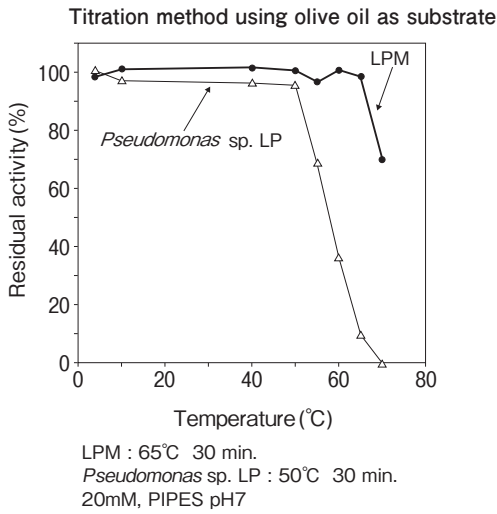


Fig.2-1 Dilution linearity using human serum

Sample : Human serum
Analyzer : Hitachi 7150

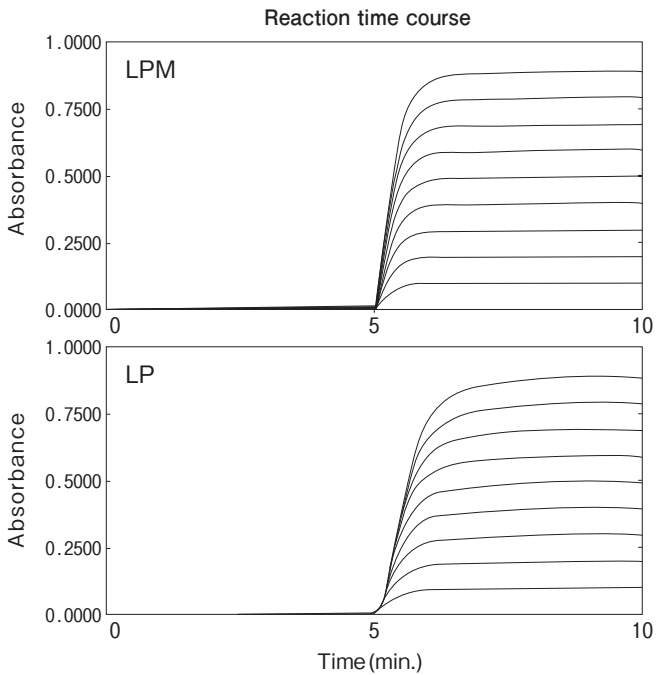


Fig.2-2 Dilution linearity using human serum

Sample : Human serum
Analyzer : Hitachi 7150

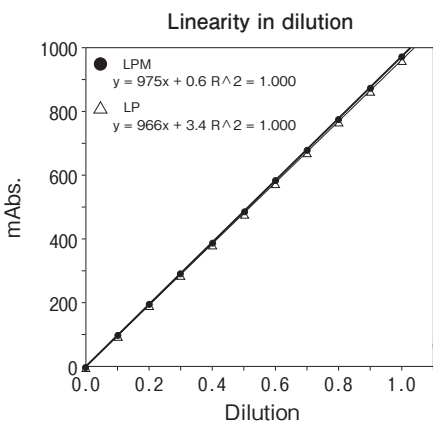


Fig.3-1 Liquid stability

Residual activity (%) after 45 days at 40°C in R2 of TG reagent

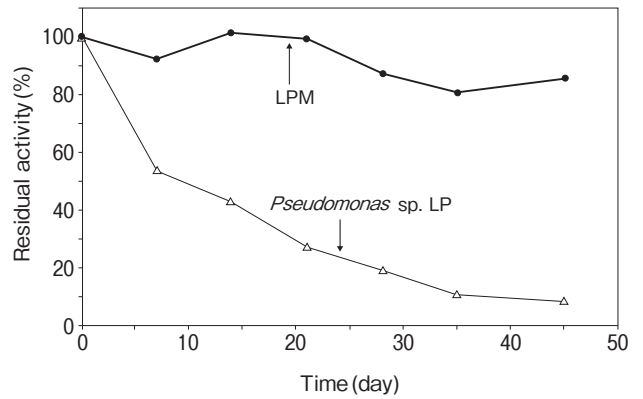
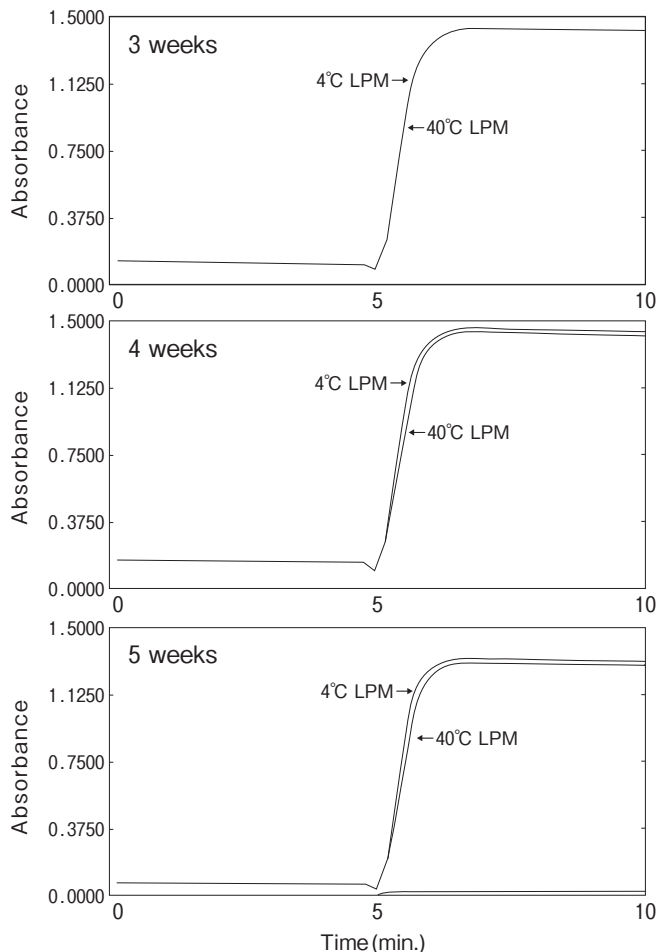


Fig.3-2 Liquid stability(Reactivity to TG)

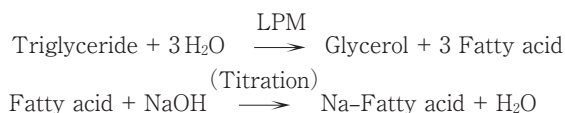
Reactivity after 3, 4 and 5 weeks at 40°C in R2 of TG reagent. (Control : Reagent stored at 4°C)



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

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. \times 200 mmH) and mix to start the preincubation at 37°C.

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

$$\begin{array}{l} \text{Titration volume} \quad \text{sample} : V_s \\ \quad \quad \quad \quad \quad \text{blank} : V_c \\ \Delta V = (V_s - V_c) \leq 2.5 \text{ ml} \\ V_c \leq 0.6 \text{ ml} \end{array}$$

■ 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. (Tokyo)*, **80**, 367-370.
6. Saiki, T., Takagi, Y., Suzuki, T., Narasaki, T., Tamura, G. and Arima, K. (1969) *Agric. Biol. Chem.*, **33**, 414.

LPM 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ル SO-120 の懸濁液)
「局方」オリーブ油 50.0g とアデカトル 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、酵素溶解希釈溶液に溶解して全容 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 0.50ml 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 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。

$$\Delta V = (V_s - V_c) \leq 2.5 \text{ ml}$$

$$V_c \leq 0.6 \text{ ml}$$

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)