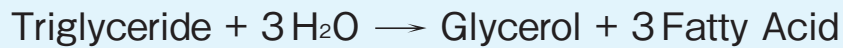


(Diagnostic Reagent Grade)

T-116

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 : More than 1,500 U/mg solid

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).

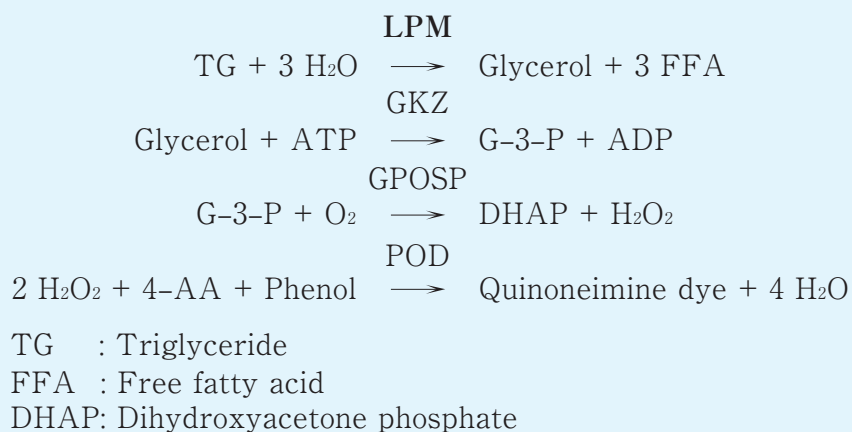


Fig.1 Thermal stability
 Stored in R2 of TG reagent
 Residual activity(%) after 30 min. at each temperature
 Activity assay method: 37°C

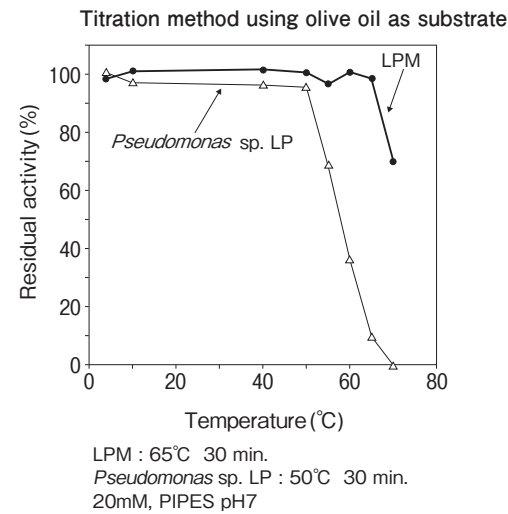


Fig.2-1 Dilution linearity using human serum
 Sample : Human serum
 Analyzer : Hitachi 7150

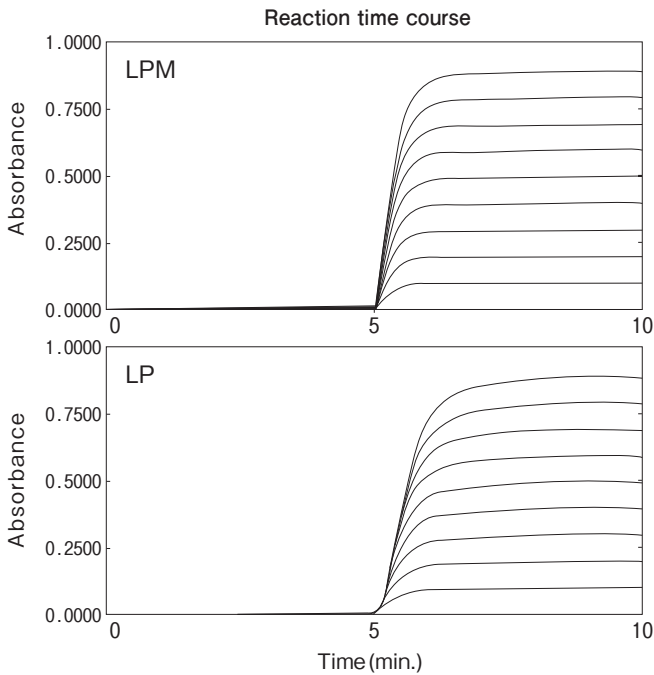


Fig.2-2 Dilution linearity using human serum
 Sample : Human serum
 Analyzer : Hitachi 7150

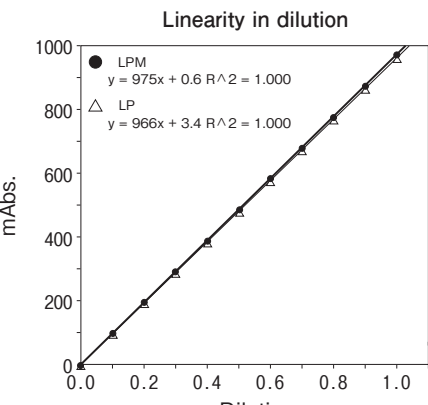


Fig.3-1 Liquid stability
 Residual activity(%) after 45 days at 40°C in R2 of TG reagent

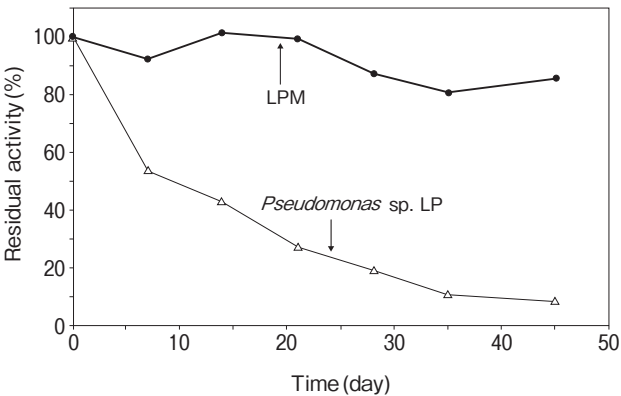
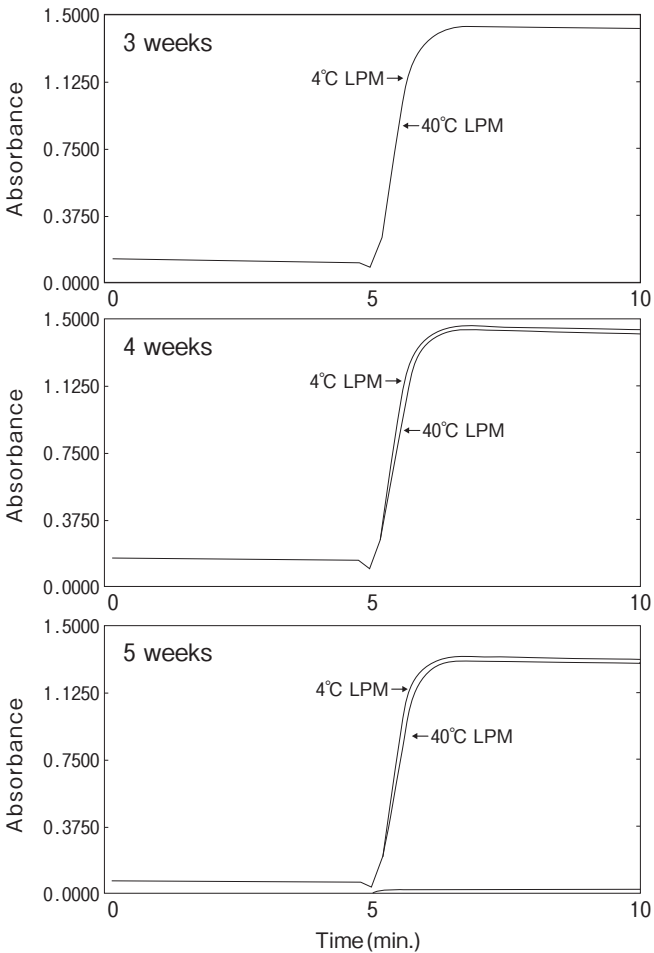


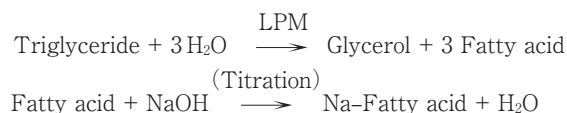
Fig.3-2 Liquid stability(Reactivity to TG)
 Reactivity after 3, 4 and 5 weeks at 40°C in R2 of TG reagent. (Control : Reagent stored at 4°C)



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 μmole 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

Titration volume sample : V_s
 blank : V_c

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

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

■ 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. (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) フェノールフタレイン-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)