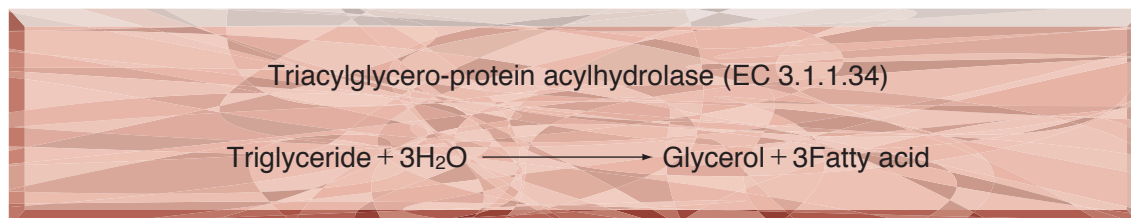


● **TOYOBO ENZYMES** ●
(Diagnostic Reagent Grade)

LIPOPROTEIN LIPASE

from Microorganism



PREPARATION and SPECIFICATION

Appearance	: Light brown amorphous powder, lyophilized
Activity	: Grade III 1.0U/mg-solid or more
Contaminants	: Phosphatase $\leq 1.0 \times 10^{-3}\%$
	: Catalase $\leq 2.0 \times 10^{-2}\%$
	: NADH oxidase $\leq 1.0 \times 10^{-3}\%$
	: Cholesterol oxidase $\leq 2.0 \times 10^{-3}\%$
Stabilizers	: Mg^{++} , BSA

PROPERTIES

Stability	: Stable at -20°C for at least one year	(Fig.1)
Inhibitors	: Hg^{++} , Cu^{++}	
Optimum pH	: 8.0	(Fig.2)
Optimum temperature	: $40-45^{\circ}\text{C}$	(Fig.3)
pH Stability	: pH 5.5–10.0 (25°C , 20hr)	(Fig.4)
Thermal stability	: below 40°C (pH 7.0, 15min)	(Fig.5)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of triglyceride in serum when coupled with L- α -glycerophosphate oxidase (G3O-321) and glycerol kinase (GYK-301, GYK-311). Usually, the reaction can be completed in 5 minutes at 37°C by using 2.5~3.0 units of the enzyme per test (3.0ml) at pH around 7.0.

ASSAY

Principle:



The appearance of quinoneimine dye is measured at 545nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of glycerol (half a micromole of quinoneimine dye) per minute under the conditions described below.

Method:

Reagents

- A. Olive oil emulsion : Sonicate the mixture of 5.0g of olive oil [reagent grade (highly refined, low acidity)] , 2.0ml of ethanol and 5.0ml of Triton X-100 solution (B) for 10 minutes (20KHz). To the oil emulsion, add 25ml of 4.0% BSA solution (C) and 15ml of 0.1M K-phosphate buffer, pH 7.0 (D), and mix. (should be prepared fresh)
- B. Triton X-100 solution : 5.0% (5.0ml Triton X-100/100ml of H₂O)
- C. BSA solution : 4.0% [4.0g bovine serum albumin/100ml of H₂O]
- D. K-phosphate buffer, pH 7.0 : 0.1M
- E. TCA solution : 0.2M (33g trichloroacetic acid/1,000ml of H₂O)
- F. MES-NaOH buffer : 50mM MES buffer, pH 6.5 [Dissolve 9.76g of 2-(N-morpholino)-ethanesulfonic acid (MW=195.23) in ca. 850ml of H₂O and, after adjusting the pH to 6.5 with 5.0N NaOH, fill up to 1,000ml with H₂O]
- G. Color developing reagent : Dissolve the following chemicals and enzymes into 200ml of 50mM MES buffer (F) in the following order:
- 4.0 ml Triton X-100 solution (B)
 - 0.04 ml N,N-Diethyl-m-toluidine (Stir until completely dissolved)
 - 4.0 mg 4-Aminoantipyrine
 - 24.2 mg ATP · Na₂ · 3H₂O
 - 40.7 mg MgCl₂ · 6H₂O
 - 200 units Glycerol kinase (Toyobo, Grade III)
 - 500 units L-α-Glycerophosphate oxidase (Toyobo, Grade III)
 - 300 units Peroxidase (Purpurogallin units)(Toyobo, Grade III)
- (Stable for one week if stored at 4°C in a brownish bottle)
- H. Enzyme diluent : 20mM K-phosphate buffer, pH 7.5 containing 2.0mM MgCl₂ and 0.5mM EDTA-Na₃

Procedure

(1st step)

- Pipette 2.0ml of olive oil emulsion (A) into a test tube and equilibrate at 37°C for about 5 minutes.
- Add 0.2ml of the enzyme solution* and mix.
- After exactly 15 minutes at 37°C, add 2.0ml of TCA solution (E) to stop the reaction and remove the precipitate by filtration through filter paper.

(2nd step)

- Pipette 0.05ml of the filtrate thus obtained into a test tube.
- Add 3.0ml of color developing reagent (G) and incubate at 37°C for further 15 minutes.
- Measure the optical density at 545nm against water (OD test).

At the same time, prepare the blank by first mixing 2.0ml of the olive oil emulsion (A) after 15min-incubation at 37°C with 2.0ml of TCA solution, followed by addition of the enzyme solution (1st step). By using the filtrate obtained from the mixture, carry out the 2nd step by the same procedure as test and measure the optical density at 545nm (OD blank).

- * Dissolve the enzyme preparation in ice-cold enzyme diluent (H) and dilute to 0.9–1.6U/ml with the same buffer, immediately before assay.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta \text{OD (OD test - OD blank)} \times \text{Vt-1} \times \text{Vt-2} \times \text{df}}{28.2 \times 1/2 \times 1.0 \times \text{t} \times \text{Vs-1} \times \text{Vs-2}} = \Delta \text{OD} \times 6.057 \times \text{df}$$

$$\text{Weight activity (U/mg)} = (\text{U/ml}) \times 1/\text{C}$$

Vt-1 : Total volume in 1st step (4.2ml)

Vt-2 : Total volume in 2nd step (3.05ml)

Vs-1 : Sample volume in 1st step (0.2ml)

Vs-2 : Sample volume in 2nd step (0.05ml)

28.2 : Millimolar extinction coefficient of quinoneimine dye under the assay condition (cm²/micromole)

1/2 : Factor based on the fact that one mole of H₂O₂ produces half a mole of quinoneimine dye

1.0 : Light path length (cm)

t : Reaction time in 1st step (15 minutes)

df : Dilution factor

C : Enzyme concentration in dissolution (c mg/ml)

Concentration in assay mixture	
K-Phosphate buffer	29.1 mM
Olive oil	90.9mg/ml
MgCl ₂	0.18 mM
Triton X-100	9.1 %
EDTA	45 μM
BSA	1.8 %

REFERENCES

- 1) T.Saiki, Y.Takagi, T.Suzuki, T.Narasaki, G.Tamura and K.Arima; *Agric. Biol. Chem.* (Tokyo), **33**, 414 (1969).
- 2) T.Yamaguchi, N.Muroya, M.Isobe and M.Sugiura; *Agric. Biol. Chem.* (Tokyo), **37**, 999 (1973).

Table 1. Effect of Various Chemicals on Lipoprotein lipase
[The enzyme (8 U/ml) was incubated at 25°C for 1 hr with each chemical.]

Chemical	Concn.(mM)	Residual activity(%)	Chemical	Concn.(mM)	Residual activity(%)
None	—	100	PCMB	2.0	91
Metal salt			MIA	2.0	95
CaCl ₂	2.0	103	NaF	20.0	101
Ba(OAc) ₂	2.0	93	NaN ₃	20.0	100
FeCl ₃	2.0	97	EDTA	5.0	88
CoCl ₂	2.0	98	o-Phenanthroline	2.0	100
MnCl ₂	2.0	82	α, α' -Dipyridyl	2.0	96
Zn(OAc) ₂	2.0	100	Borate	20.0	99
NiCl ₂	2.0	99	Triton X-100	1.0%	84
Pb(OAc) ₂	2.0	76	Brij 35	1.0%	99
AgNO ₃	2.0	94	SDS	0.1%	91
HgCl ₂	2.0	58	Tween 20	0.1%	98
CdCl ₂	1.0	101	Span 20	0.1%	101
CuSO ₄	1.0	1.5	Na-cholate	1.0%	99
NEM	2.0	101	Taurocholate	0.1%	100

Ac, CH₃CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediaminetetraacetate; NEM, N-Ethylmaleimide; SDS, Sodium dodecyl sulfate; DAC, Dimethylbenzylalkylammonium chloride.

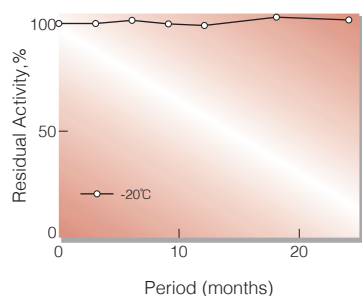


Fig.1. Stability (Powder form)
[kept under dry conditions]

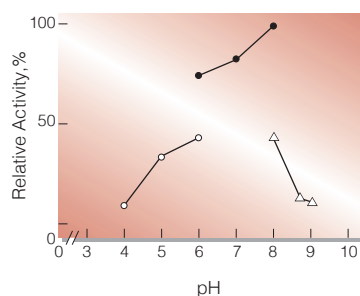


Fig.2. pH-Activity
[37°C in 0.1M buffer solution:
pH4.2-6.0, acetate; pH6.0-8.0,
K-phosphate; pH8.0-9.0, Tris-HCl]

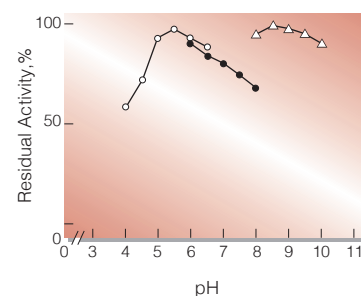


Fig.4. pH-Stability
[25°C, 20hr-treatment with 0.1M
buffer solution: pH4.0-6.5, acetate;
pH6.0-8.0, K-phosphate; pH8.0-10.0
Tris-HCl]

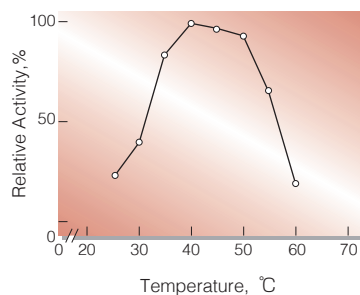


Fig.3. Temperature activity
[in 0.1M K-phosphate buffer, pH7.0]

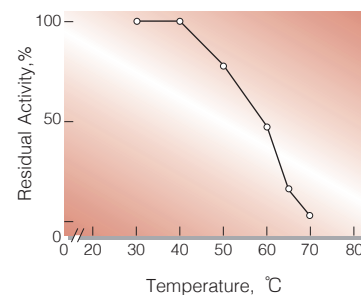


Fig.5. Thermal stability
[15min-treatment with 0.1M K-phosphate, pH7.0]
enzyme concn.:20 U/ml]

活性測定法 (Japanese)

1.原理

Triglyceride + 3H₂O $\xrightarrow{\text{lipoprotein lipase}}$ Glycerol + 3Fatty acid

Glycerol + ATP $\xrightarrow[\text{Mg}^{++}]{\text{glycerol kinase}}$ Glycerol-3-P + ADP

Glycerol-3-P + O₂ $\xrightarrow{\text{L-}\alpha\text{-glycerophosphate oxidase}}$

Dihydroxyacetone-P + H₂O₂

2H₂O₂ + 4-Aminoantipyrine + N,N-Diethyl-m-toluidine $\xrightarrow{\text{peroxidase}}$ Quinoneimine dye + 4H₂O

4-AminoantipyrineとN,N-Diethyl-m-toluidineの酸化縮合生成物であるQuinoneimine色素を545nmで測定し、上記反応で生成したH₂O₂を定量する。

2.定義

下記条件で1時間に1マイクロモルのGlycerol (1/2マイクロモルのQuinoneimine色素)を生成する酵素量を1単位 (U)とする。

3.試薬

- A. オリーブ油エマルジョン液 [オリーブ油 (ナカライテスク製, 臨床化学研究用) 5.0gとエタノール2.0mlと5.0%トリトンX-100溶液(B) 5.0mlの混合液を10分間超音波処理し (20KHz), エマルジョンを調製する。次いでこのエマルジョンに4.0% BSA水溶液 (C) 25mlと0.1MK-リン酸緩衝液, pH7.0 (D) 15mlを添加混合する] (用時調製)
- B. 5.0%(V/V)トリトンX-100溶液(5.0mlのTriton X-100(純正)を100mlの蒸留水に溶解する)
- C. 4.0%牛血清アルブミン(BSA)水溶液 [4.0gの牛血清アルブミンを100mlの蒸留水に溶解する]
- D. 0.1MK-リン酸緩衝液, pH7.0
- E. 0.2Mトリクロル酢酸(TCA)溶液(33gのトリクロル酢酸を1,000mlの蒸留水に溶解する)
- F. 50mM MES緩衝液, pH6.5 [9.76gの2-(N-morpholino) ethanesulfonic acid (MW=195.23)を約850mlの蒸留水に溶解し, pHを5.0N NaOHで6.5に調整後, 蒸留水で1,000mlとする]
- G. 発色試薬 [200mlの50mM MES緩衝液, pH6.5(F)に下記順序で試薬及び酵素を溶解する]

4.0 ml	5.0%トリトンX-100溶液 (B)
0.04 ml	N,N-Diethyl-m-toluidine (完全に溶解するまで攪拌する)
4.0 mg	4-アミノアンチピリン
24.2 mg	ATP · Na ₂ · 3H ₂ O
40.7 mg	MgCl ₂ · 6H ₂ O
200 単位	Glycerol kinase (東洋紡製, Grade III)
500 単位	L-α-Glycerophosphate oxidase (東洋紡製, Grade III)
300 単位	Peroxidase (プルプロガリン単位) (東洋紡製, Grade III)

(上記発色試薬は4°C, 褐色瓶中で保存すれば1週間は使用可能)

酵素溶液: 酵素標品を予め氷冷した2.0mM MgCl₂及び0.5mM EDTA-Na₃を含む20mMK-リン酸緩衝液, pH7.5で溶解し, 分析直前に同緩衝液で0.9~1.6U/mlに希釈する。

4.手順

- ① オリーブ油エマルジョン液(A) 2.0mlを試験管に採り, 37°Cで約5分間予備加温する。
- ② 酵素溶液0.2mlを加え, 反応を開始する。
- ③ 37°Cで正確に15分間反応させた後, TCA溶液 (E) 2.0mlを加えて反応を停止する。
- ④ 生成する不溶物を濾紙濾過で除く。
- ⑤ 濾液の0.05mlを試験管に採り, 発色試薬(G) 3.0mlを加えて混合した後, 37°Cにて15分間加温し, 545nmにおける吸光度を測定する(OD test)。
- ⑥ 盲検はオリーブ油エマルジョン液(A) 2.0mlを37°Cで15分間放置後, TCA溶液(E) 2.0mlを加え, 次いで酵素溶液0.2mlを加えて調製し, 以下上記同様 (④~⑤)に操作して吸光度を測定する(OD blank)。

5.計算式

$$U/ml = \frac{\Delta OD (OD \text{ test} - OD \text{ blank}) \times 4.2(\text{ml}) \times 3.05(\text{ml}) \times \text{希釈倍率}}{28.2 \times 1/2 \times 1.0 \times 15(\text{分}) \times 0.2(\text{ml}) \times 0.05(\text{ml})}$$

$$= \Delta OD \times 6.057 \times \text{希釈倍率}$$

$$U/mg = U/ml \times 1/C$$

28.2 : Quinoneimine色素の上記測定条件下でのミリモル分子吸光度係数(cm²/micromole)

1/2 : H₂O₂の1分子から形成するQuinoneimine色素は1/2分子である事による係数

1.0 : 光路長(cm)

C : 溶解時の酵素濃度(c mg/ml)