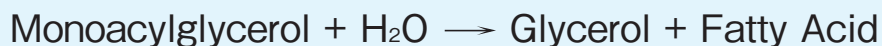


(Diagnostic Reagent Grade)

T-117

MONOGLYCERIDE LIPASE [MGLP II]

from *Bacillus* sp.
(Glycerol-ester hydrolase, EC 3.1.1.23)



Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 20 U/mg solid
Contaminants :
Catalase : Less than 0.5% (U/U)

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 20 kDa (gel filtration)	
Isoelectric point	: pH 4.8 ± 0.2	
Michaelis constant	: Monolaurine 1.8 × 10 ⁻⁴ M	
Optimum pH	: 6.0–8.0	Figure 1
pH stability	: 6.0–8.0 (65°C, 10 min)	Figure 2
Optimum temperature	: 65°C (PIPES buffer)	Figure 3
Thermal stability	: Stable at 65°C and below (pH 8.0, 10 min)	Figure 4
Effect of metal ions	: See Table 2	
Effect of detergents	: See Table 3	

Applications for Diagnostic Test

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



TG: Triglyceride
FFA: Free fatty acid

Table 1. Substrate specificity

Substrate	Relative activity (%)
1-Monocaprylin	81.8
1-Monolaurin	100
1-Monomyristin	96.3
1-Monopalmitin	66.3
1-Monostearin	31.0
1-Monoolein	62.3
1-Monolinolein	110
Triolein	0

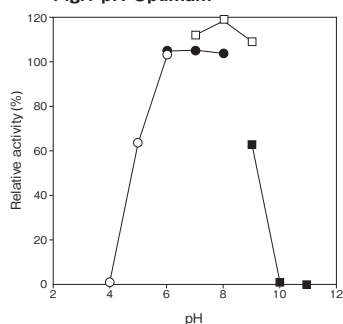
Table 2. Effect of metal ions on MGLP II activity

Metal ion (10mM)	Relative activity (%)
None	100
NaCl	83.0
KCl	79.0
LiCl	78.0
MgCl ₂	77.0
MnCl ₂	77.0
CaCl ₂	78.0

Table 3. Effect of detergents on MGLP II activity

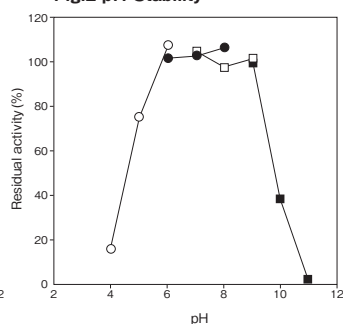
Detergent (0.1%)	Relative activity (%)
None	100
Triton X-100	67.0
Triton X-114	67.0
Adekanol 795	64.0
Emulgen B-66	67.0
Emulgen 911	65.0
Emulgen 810	66.0
Emulgen 460	61.0
Rheodol TWL-106	67.0

Fig.1 pH Optimum



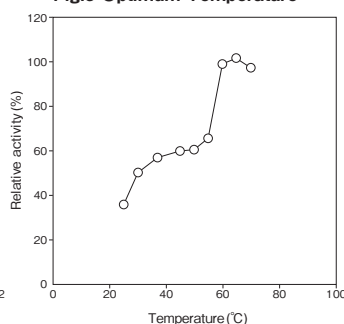
○ : Acetate buffer
 ● : Phosphate buffer
 □ : Tris-HCl buffer
 ■ : Glycine-NaOH

Fig.2 pH Stability



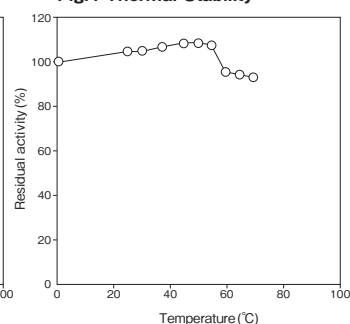
65°C, 10min
 ○ : 3,3-Dimethylglutarate-NaOH buffer
 ● : PIPES buffer
 □ : Tris-HCl buffer
 ■ : Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 7.3
 50 mM PIPES buffer

Fig.4 Thermal Stability

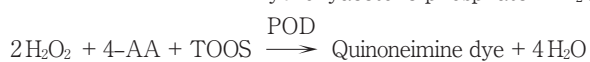
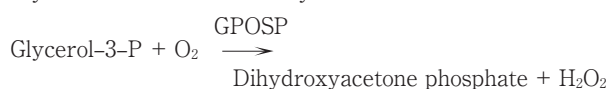


pH 8.0, 10 min.
 50 mM PIPES buffer

Assay

Principle

The assay is based on the increase in absorbance at 550 nm as the formation of quinoneimine dye proceeds in the following reactions:



ATP: Adenosine triphosphate

GK: Glycerol kinase

GPOSP: Glycerophosphate oxidase

TOOS: Ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine sodium salt,

Unit definition

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

Reagents

1. Reaction mixture	
0.2 M PIPES-NaOH buffer pH 7.3	0.10 ml
15mM 4-AA solution	0.05 ml
0.3% (W/V) TOOS solution	0.05 ml
100 U/ml POD solution ¹⁾	0.025 ml
100 mM MgCl ₂ solution	0.005 ml
50 mM ATP solution pH7.0	0.01 ml
25 U/ml GK solution ²⁾	0.01 ml

150 U/ml GPOSP solution ³⁾ 0.10 ml
 Distilled water 0.05 ml
 1): 100 U/ml POD solution
 Dissolve 1,000 U (PPU) of POD with 10 ml of distilled water.
 2): 25 U/ml GK solution
 Dissolve 250 U of GK with 10 ml of distilled water.
 3): 150 U/ml GPOSP solution
 1,500 U of GPOSP with 10 ml of distilled water.

2. Substrate solution
 (1) Substrate preparation buffer
 5 mM MES-NaOH buffer pH 5.5 containing 0.5% (W/V) Triton X-100
 MES [2-(N-monophoryno)ethanesulfonic acid monohydrate]
 (2) Substrate solution (for stock)
 0.5M Monolaurine-ethanol solution
 (3) Substrate solution (milky colored)
 Mix 0.2 ml of substrate solution (for stock) and 9.8 ml of substrate preparation buffer

3. Reaction stopper
 0.5% (W/V) SDS solution
 SDS: Sodium dodecyl sulfate

4. Enzyme dilution buffer
 10 mM PIPES-NaOH buffer pH 7.3 containing 0.1% (W/V) BSA

5. Reagents
 PIPES [Piperazine-1,4-bis (2-ethanesulfonic acid)]:
 Dojindo Laboratories #345-02225
 TOOS: Dojindo Laboratories #OC13
 MES: Dojindo Laboratories #349-01623
 BSA: Millipore Fraction V pH5.2 #81-053
 Monolaurin: Tokyo Kasei Kogyo Co., Ltd #G0081
 GK: Nagase Diagnostics Co., Ltd. #T-09
 GPOSP: Nagase Diagnostics Co., Ltd. #T-60
 4-AA: NACALAI TESQUE, INC. Special grade #01907-52
 Triton X-100: The Dow Chemical Company
 SDS: NACALAI TESQUE, INC. #316-06
 POD: Sigma Chemical Co. Type II #P-8250

Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

Procedure

1. Pipette accurately 0.40 ml of reaction mixture and 50 μ l of substrate solution into a small test tube and preincubate at 37°C.
2. After 3 min, add exactly 20 μ l of enzyme solution and mix to start the reaction at 37°C.
 ※ In the case of a test blank, add 20 μ l of enzyme dilution buffer in place of enzyme solution.
3. At 10 minutes after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 550 nm.

Absorbance sample : As
 blank : Ab

$$\Delta A = (A_s - A_b) \leq 0.700 A_b$$

Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A/10}{15.6} \times \frac{2.47}{0.02} \times \frac{1}{X}$$

15.6 : millimolar extinction coefficient of quinoneimine dye at 550 nm ($\text{cm}^2 / \mu\text{mole}$)

10 : reaction time (min)

2.47 : final volume (ml)

0.02 : 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.

Reference

Imamura, S., and Kitaura, S. (2000) J. Biochem. (Tokyo), 127, 419-425.

MGLP II 活性測定法 (Japanese)

I. 試薬液

1. 反応試薬混合液

0.2M PIPES-NaOH 緩衝液 pH7.3 0.10 ml
 15mM 4-AA 溶液 0.05 ml
 0.3% (W/V) TOOS 溶液 0.05 ml
 100U/ml POD 溶液 ¹⁾ 0.025 ml
 100mM 塩化マグネシウム溶液 0.005 ml
 50mM ATP 溶液 pH7.0 0.01 ml
 25U/ml GK 溶液 ²⁾ 0.01 ml
 150U/ml GPOSP 溶液 ³⁾ 0.10 ml
 精製水 0.05 ml

1): 100U/ml POD 溶液

POD 1,000 単位 (PPU) を精製水 10ml で溶解する。

2): 25U/ml GK 溶液

GK 250 単位 (U) を精製水 10ml で溶解する。

3): 150U/ml GPOSP 溶液

GPOSP 1,500 単位 (U) を精製水 10ml で溶解する。

2. 基質溶液

① 基質調製用液

0.5% (W/V) トリトン X-100 を含む 5mM MES-NaOH 緩衝液 pH5.5

② 保存基質溶液

0.5M モノラウリン-エタノール溶液

③ 基質溶液

保存基質溶液 0.2ml と基質調製用液 9.8ml を混合（白濁する）して基質溶液とする。

3. 反応停止液

0.5% (W/V) SDS 溶液

4. 酵素溶解希釈用液

0.1% (W/V) BSA を含む 10mM PIPES-NaOH 緩衝液 pH7.3

5. 試薬

PIPES [ピペラジン-1,4-ビス (2-エタンスルホン酸)] : 同仁化学製 #345-02225

TOOS [エチル-N-(2-ヒドロキシ-3-スルホプロピル)-m-トリイジンナトリウム塩] : 同仁化学製 #OC13

MES [2-(N-モルフォリノ) エタンスルホン酸モノヒドレート] : 同仁化学製 #349-01623

ATP (アデノシン三リン酸・2Na・2H₂O) :

協和発酵
製BSA : Millipore 製 Fraction V pH5.2 #81-053

モノラウリン (Monolaurin) :

東京化成工業製 #G0081

GK (グリセロールキナーゼ) :

ナガセダイアグノスティックス製 #T-09

GPOSP (グリセロリン酸オキシダーゼ) :

ナガセダイアグノスティックス製 #T-60

4-AA : ナカライテスク製 特級 #01907-52

トリトン X-100 : Dow Chemical 製

SDS (ドデシル硫酸ナトリウム) :

ナカライテスク製 #316-06

POD : シグマ製 Type II #P-8250

II. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液で溶解して全容 20ml 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. 小試験管に反応試薬混合液 0.40ml と基質溶液 50 μ l を正確に分注し、37℃で予備加温する。

2. 3 分経過後、酵素試料液 20 μ l を正確に加えて混和し、37℃で反応を開始する。

※盲検は酵素試料液の代りに酵素溶解希釈用液 20 μ l を加える。

3. 10 分経過後、反応停止液 2.0ml を加えて混和し、反応を停止する。

4. 550nm における吸光度を測定する。

求められた吸光度変化を試料液は As、盲検液は Ab とする。

$$\Delta A = (A_s - A_b) \leq 0.700 A_{bs}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A / 10}{15.6} \times \frac{2.47}{0.02} \times \frac{1}{X}$$

15.6 : キノン色素の 550nm におけるミリモル分子

吸光係数 (cm²/ μ mole)

10 : 反応時間 (min)

2.47 : 反応総液量 (ml)

0.02 : 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)