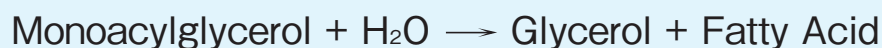


# MONOGLYCERIDE LIPASE [MGLP II]

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  $1.8 \times 10^{-4}\text{M}$   
 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

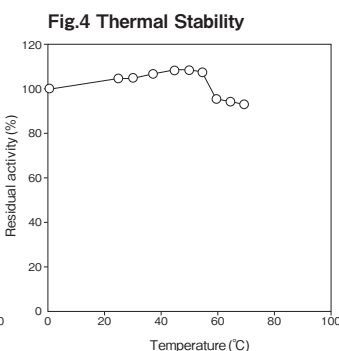
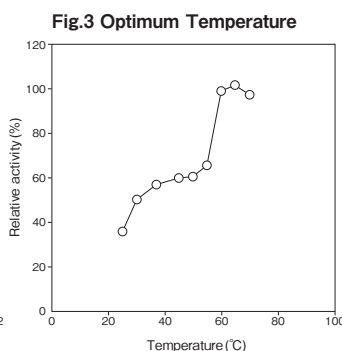
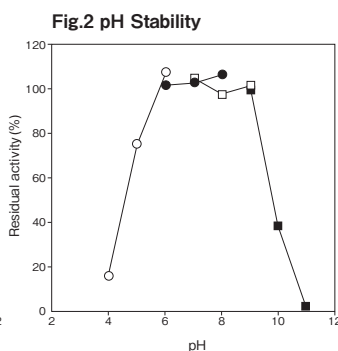
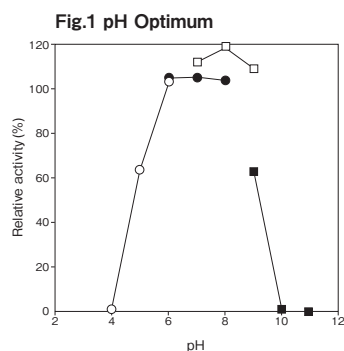
Substrate	Relative activity (%)
1-Monocaprylin	81.8
1-Monolaurin	100
1-Monomyristin	96.3
1-Monopalmitin	66.3
1-Monostearin	31.0
1-Monoolein	62.3
1-Monolinolein	110
Triolein	0

Table 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

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 <sub>2</sub>	77.0
MnCl <sub>2</sub>	77.0
CaCl <sub>2</sub>	78.0



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

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

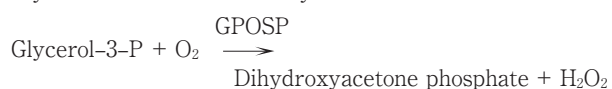
pH 7.3  
50 mM PIPES buffer

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 <sup>1)</sup>	0.025 ml
100 mM MgCl <sub>2</sub> solution	0.005 ml
50 mM ATP solution pH7.0	0.01 ml
25 U/ml GK solution <sup>2)</sup>	0.01 ml

- 150 U/ml GPOSP solution <sup>3)</sup> 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: Asahi Kasei Pharma Corporation #T-09  
 GPOSP: Asahi Kasei Pharma Corporation #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

- Pipette accurately 0.40 ml of reaction mixture and 50  $\mu$ l of substrate solution into a small test tube and preincubate at 37°C.
- After 3 min, add exactly 20  $\mu$ l of enzyme solution and mix to start the reaction at 37°C.
- At 10 minutes after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
- 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 <sup>1)</sup>	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 <sup>2)</sup>	0.01 ml
150U/ml GPOSP 溶液 <sup>3)</sup>	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・2H<sub>2</sub>O) :

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) : 旭化成ファーマ製

#T-09

GPOSP (グリセロリン酸オキシダーゼ) :

旭化成ファーマ製 #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  $\mu$ l を正確に分注し、37°C で予備加温する。

2. 3分経過後、酵素試料液 20  $\mu$ l を正確に加えて混和し、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 20  $\mu$ 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 (cm<sup>2</sup>/  $\mu$ 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)