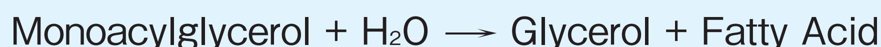
MONOGLYCERIDE LIPASE [MGLP Ⅱ]

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


Monoacylglycerol + H_2O ⟶ Glycerol + Fatty Acid

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℃, 10 min)	Figure 2
Optimum temperature	: 65℃ (PIPES buffer)	Figure 3
Thermal stability	: Stable at 65℃ 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**.

LP + **MGLP** Ⅱ
TG + 3 H_2O ⟶ Glycerol + 3 FFA

TG: Triglyceride
FFA: Free fatty acid

Table 1. Substrate specificity

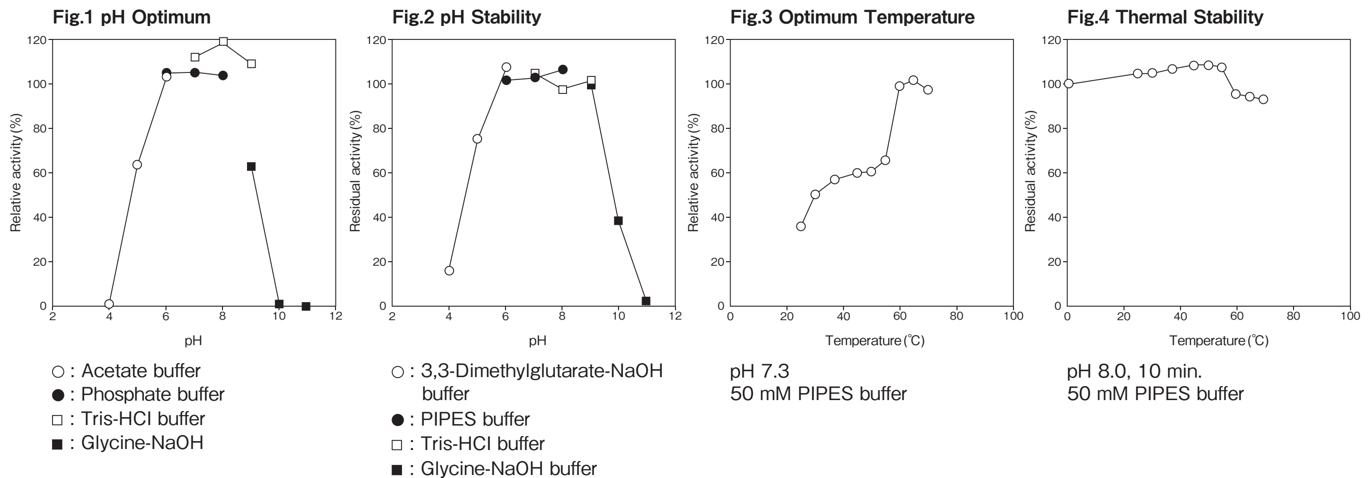
Substrate	Relative activity (%)
1-Monocaprylin	81.8
1-Monolaurin	100
1-Monomyristin	96.3
1-Monopalmitin	66.3
1-Monostearin	31.0
1-Monoolein	62.3
1-Monolinolein	110
Triolein	0

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

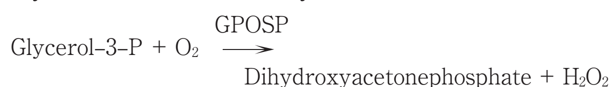
Detergent (0.1%)	Relative activity (%)
None	100
Triton X-100	67.0
Triton X-114	67.0
Adekanol 795	64.0
Emulgen B-66	67.0
Emulgen 911	65.0
Emulgen 810	66.0
Emulgen 460	61.0
Rheodol TWL-106	67.0



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℃ 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: [Pyperazine–N,N'–bis (2–ethanesulfonic acid)] Dojindo Laboratories #345–02225
TOOS: Dojindo Laboratories
MES: Dojindo Laboratories
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

1. Pipette accurately 0.40 ml of reaction mixture and 50 μl of substrate solution into a small test tube and preincubate at 37℃.
2. After 3 min, add exactly 20 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 20 μl 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

$\triangle A = (As - Ab) \leqq 0.700Abs$

■ Calculation

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

15.6 : millimolar extinction coefficient of quinoneimine dye at 550 nm (cm^2/ μmole)
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℃ 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-N,N'-ビス（2-エタンスルフォン酸）］：同仁化学製　#345-02225
TOOS［エチル-N-（2-ヒドロキシ-3-スルフォプロピル）-m-トルイジンナトリウム塩］：同仁化学製
MES［2-（N-モルフォリノ）エタンスルフォン酸モノヒドレート］：同仁化学製
ATP（アデノシン三リン酸･2Na･$2H_2O$）：協和発酵製
BSA : Millipore 社製　Fraction V pH5.2 #81-053
モノラウリン（Monolaurin）：東京化成工業社製　#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：ナカライテスク社製　特級　#01907-52
トリトン X-100：Dow Chemical 社製
SDS（ドデシル硫酸ナトリウム）：ナカライテスク社製　#316-06
POD：シグマ社製　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 μl 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 μl 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 μl 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。

$$\Delta A = (As - Ab) \leqq 0.700Abs$$

Ⅳ．計算

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

15.6 : キノン色素の 550nm におけるミリモル分子吸光数（$cm^2/\mu mole$）
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）