INQUIRY NEEDED

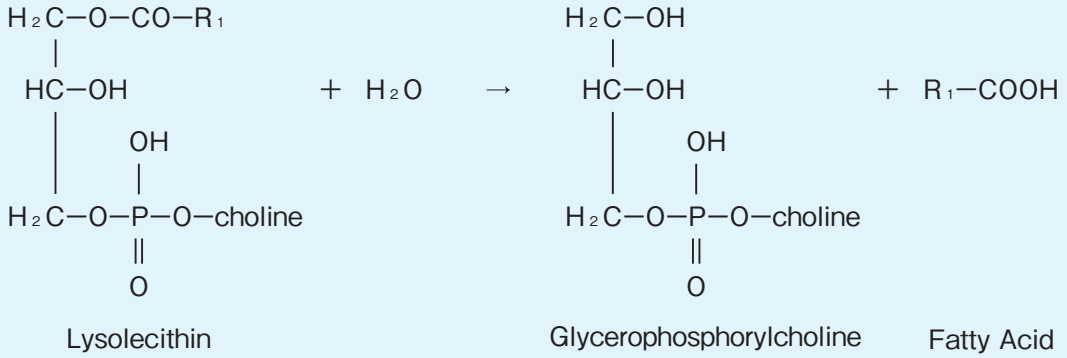
(Diagnostic Reagent Grade)

ASAHI KASEI ENZYMES T-32

LYSOPHOSPHOLIPASE [LYPL]

from *Vibrio* sp.

(2-Lysophosphatidylcholine acylhydrolase, EC 3.1.1.5)



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Preparation and Specification

Appearance : White to brownish amorphous powder, lyophilized
Specific activity : More than 20 U/mg solid

Properties

Michaelis constant	: Lysolecithin 6.7×10^{-4} M	
Optimum pH	: 9.0–9.5	Figure 1
pH stability	: 6.5–9.0 (37℃, 60 min)	Figure 2
Thermal stability	: Stable at 60℃ and below (pH 7.2, 10 min)	Figure 3
Effect of various chemicals	: See Table 1 and Table 2	
Activator	: Ca^{2+}	
Inhibitors	: Cationic detergents	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **lysolecithin** when coupled with glycerophosphorylcholine phosphodiesterase (T-33) and choline oxidase (T-05).

$$\text{Lysolecithin} + H_2O \xrightarrow{\text{LYPL}} \text{Glycerophosphorylcholine} + \text{Fatty acid}$$

$$\text{Glycerophosphorylcholine} + H_2O \xrightarrow{\text{GPCP}} \text{Glycerophosphate} + \text{Choline}$$

$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{\text{COD}} \text{Betaine} + 2\,H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

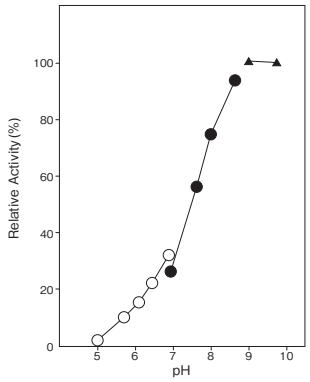
Table 1. Effect of detergents on lysophospholipase activity

Detergent	Concentration (%)	Relative activity (%)
None	–	100
Triton X-100	0.5	119
Adekatol SO-145	0.5	42
Adekatol PC-8	0.5	74
Adekatol NP-700	0.5	103
Pluronic L-61	0.5	119
Sodium cholate	0.5	117
Cethyl pyridinium chloride	0.5	0
Cethyl trimethyl-Ammonium chloride	0.5	0

Table 2. Effect of metal ions on lysophospholipase activity

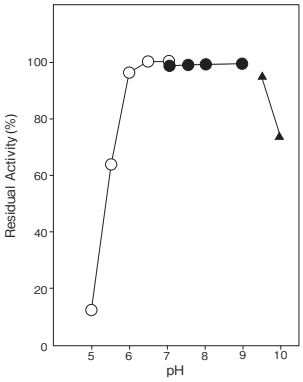
Metal ion	Concentration (mM)	Relative activity (%)
None	–	100
$CaCl_2$	1	119
$MgCl_2$	1	115
$MnCl_2$	1	130
$ZnCl_2$	1	14
$BaCl_2$	1	127
$CoCl_2$	1	117
$CuCl_2$	1	9
$NiCl_2$	1	110
NH_4Cl	100	71
NaCl	100	89
KCl	100	81

Fig.1 pH Optimum



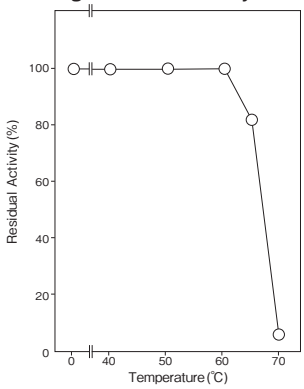
○ : 3,3-Dimethylglutarate -NaOH buffer
● : Tris-HCl buffer
▲ : Borate buffer

Fig.2 pH Stability



37℃, 60 min.
○ : 3,3-Dimethylglutarate -NaOH buffer
● : Tris-HCl buffer
▲ : Borate buffer

Fig.3 Thermal Stability



pH 7.2, 10 min.
10 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 500 nm as the formation of quinoneimine dye proceeds in the following reactions:

$$\text{Lysolecithin} + H_2O \xrightarrow{\text{LYPL}} \text{Glycerophosphorylcholine} + \text{Fatty acid}$$

$$\text{Glycerophosphorylcholine} + H_2O \xrightarrow{\text{GPCP}} \text{Glycerophosphate} + \text{Choline}$$

$$\text{Choline} + 2\,O_2 + H_2O \xrightarrow{\text{COD}} \text{Betaine} + 2\,H_2O_2$$

$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

GPCP: Glycerophosphorylcholine phosphodiesterase
COD: Choline oxidase

■ Unit definition

One unit is defined as the amount of enzyme which hydrolyzes 1 μmole of lysolecithin per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture for the first reaction

0.2 M Tris-HCl buffer pH 8.0	0.10 ml
10 mM Lysolecithin solution	0.05 ml
10 mM $CaCl_2$ solution	0.05 ml
1 U/ml GPCP solution [1)]	0.10 ml
Distilled water	0.15 ml

1): 1 U/ml GPCP solution
Dissolve 10 U of GPCP with 10 ml of 10 mM Tris-HCl buffer pH 8.0.

2. Reaction mixture for the second reaction

0.2 M Tris-HCl buffer pH 8.0	0.10 ml
0.1 M EDTA solution pH 8.0	0.20 ml
15 mM 4-AA solution	0.10 ml
0.2 % (W/V) Phenol solution	0.10 ml
60 U/ml COD solution [2)]	0.10 ml
100 U/ml POD solution [3)]	0.05 ml
Distilled water	0.35 ml

EDTA: Ethylenediamine tetraacetic acid
2): 60 U/ml COD solution
Dissolve 600 U of COD with 10 ml of 10 mM Tris-HCl buffer pH 8.0.
3): 100 U/ml POD solution
1,000 U (PPU) of POD with 10 ml of distilled water.

3. Enzyme dilution buffer
   10 mM Tris-HCl buffer pH 8.0 containing 0.05% (W/V) bovine serum albumin
4. Reagents:
   Lysolecithin (1-Oleoyl-sn-glycero-3-phosphocholine): Sigma Chemical Co. #L-1881
   GPCP: Asahi Kasei Pharma Corporation #T-33
   COD: Asahi Kasei Pharma Corporation #T-05
   4-AA: NACALAI TESQUE, INC. Special grade #01907-52
   POD: Sigma Chemical Co. Type II #P-8250
   EDTA (2Na·2H2O): KISHIDA CHEMICAL Co., Ltd. #060-29133

■ Enzyme solution

Weigh about 20 mg of test sample exactly 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 0.45 ml of reaction mixture for the first reaction into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 50 μl of enzyme solution and mix to start the first reaction at 37℃.
   ※ In the case of a test blank, add 50 μl of enzyme dilution buffer in place of enzyme solution at this point.
3. After 10 min, add immediately 1.0 ml of reaction mixture for the second reaction and mix to start the second reaction at 37℃.
4. After 20 min, add 1.5 ml of distilled water to stop the reaction.
5. Measure the absorbance at 500 nm.
   Absorbance sample : As
   blank : Ab
   △A = (As − Ab) ≦ 0.07 Abs

■ Calculation

$$\text{Activity (U/mg)} = \frac{\triangle A/10}{12.0 \times 1/2} \times \frac{1}{2} \times \frac{3.00}{0.05} \times \frac{1}{X}$$

12.0 : millimolar extinction coefficient of quinoneimine dye at 500 nm (cm^2 / μmole)
2 : a multiplier derived from the fact that 1 mole of lysolecithin produces 2 mole of H_2O_2
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye
10 : reaction time (min)
3.00 : final volume (ml)
0.05 : 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.

References

1. Misaki, H. and Matsumoto, M. (1978) J. Biochem., **83**, 1395-1405.
2. Brumley, G. and Van Den Bosch, H. (1977) J. Lipid Res., **18**, 523-533.
3. Scandella, C. J. and Kornberg, A. (1971) Biochemistry, **10**, 4447-4457.

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LYPL 活性測定法 (Japanese)

Ⅰ. 試薬液

1. 第一反応試薬混合液

0.2M トリス-HCl 緩衝液 pH8.0	0.10 ml
10mM リゾレシチン溶液	0.05 ml
10mM 塩化カルシウム溶液	0.05 ml
1U/ml GPCP 溶液 1)	0.10 ml
精製水	0.15 ml

1):1U/ml GPCP 溶液
GPCP 10 単位 (U) を 10mM トリス-HCl 緩衝液 pH8.0 10ml で溶解する。

2. 第二反応試薬混合液

0.2M トリス-HCl 緩衝液 pH8.0	0.10 ml
0.1M EDTA 溶液 pH8.0	0.20 ml
15mM 4-AA 溶液	0.10 ml
0.2% (W/V) フェノール液	0.10 ml
60U/ml COD 溶液 2)	0.10 ml
100U/ml POD 溶液 3)	0.05 ml
精製水	0.35 ml

2):60U/ml COD 溶液
COD 600 単位 (U) を 10mM トリス-HCl 緩衝液 pH8.0 10ml で溶解する。

3):100U/ml POD 溶液
POD 1,000 単位 (PPU) を精製水 10ml で溶解する。

3. 酵素溶解希釈用液
   0.05% (W/V) BSA を含む 10mM トリス-HCl 緩衝液 pH8.0
4. 試薬
   リゾレシチン (1- オレオイル -sn- グリセロ -3- ホスホコリン):シグマ製 #L-1881
   GPCP:旭化成ファーマ製 #T-33
   COD:旭化成ファーマ製 #T-05
   4-AA:ナカライテスク製 特級 #01907-52
   POD:シグマ製 Type Ⅱ #P-8250
   EDTA (エチレンジアミン四酢酸·2Na·2H2O): キシダ化学製 #060-29133

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. 小試験管に第一反応試薬混合液 0.45ml を正確に分注し、37℃で予備加温する。
2. 5 分経過後、酵素試料液 50 μl を正確に加えて混和し、37℃で第一反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μl を加える。
3. 10 分経過後、第二反応試薬混合液 1.0ml を加えて混和し、37℃で第二反応を開始する。
4. 20 分経過後、精製水 1.5ml を加えて混和し、反応を停止する。
5. 500nm における吸光度を測定する。
   求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (As - Ab) \leqq 0.07\ Abs$$

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta\ A/10}{12.0 \times 1/2} \times \frac{1}{2} \times \frac{3.00}{0.05} \times \frac{1}{X}$$

12.0 : キノンイミン色素の 500nm におけるミリモル分子吸光係数 (cm^2 / μmole)
1/2 : H_2O_2 2 モルからキノンイミン色素 1 モルが生成することによる係数
2 : リゾレシチン 1 モルから H_2O_2 2 モルが生成することによる係数
10 : 反応時間 (min)
3.00 : 反応総液量 (ml)
0.05 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液の検品濃度 (mg/ml)