L-α-GLYCEROPHOSPHATE OXIDASE [GPOSP]

from *Streptococcus* sp.

(*sn*-Glycero-3-phosphate: oxygen 2-oxidoreductase, EC 1.1.3.21)

L-α-Glycerophosphate + O_2 ⟶ Dihydroxyacetone phosphate + H_2O_2

★ Advantages
① Highly purified enzyme
② Stability in solution
③ Registance for antiseptic reagents

Preparation and Specification

Appearance : Yellowish amorphous, lyophilized
Specific activity : More than 40 U/mg solid
Contaminants :
Acetate kinase Less than 0.1 % (U/U)
Lactate oxidase Less than 0.001% (U/U)

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 180 kDa (Sephacryl S-200) 130 kDa (Sephadex G200) 67 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.03	
Michaelis constants	: L-α-Glycerophosphate 2.23 mM (pH 6.5) 4.18 mM (pH 7.5)	
Optimum pH	: 6.5 and 8.5-9.0	Figure 1
pH stability	: 5.0-7.0 (37℃, 30 min)	Figure 2
Optimum temperature	: 37℃	
Thermal stability	: Stable at 55℃ and below (100 mM Phosphate buffer pH 6.5, 5 min.)	Figure 3
Effect of various chemicals	: See Table 2	
Stabilizers	: FAD, Sucrose	
Electrophoresis pattern	: See Figure 4	
Liquid stability (Buffer pH)	: See Figure 5	
(Detergents)	: See Figure 6	
Antiseptic stability	: See Figure 7	
Turbidity test	: See Table 3	

Applications for Diagnostic Test

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

$$TG + 3\,H_2O \xrightarrow{LP} Glycerol + 3\ Fatty\ acid$$

$$Glycerol + ATP \xrightarrow{GKZ} G\text{-}3\text{-}P + ADP$$

$$G\text{-}3\text{-}P + O_2 \xrightarrow{GPOSP} DHAP + H_2O_2$$

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

TG: Triglyceride, DHAP: Dihydroxyacetone phosphate

Table 1. Substrate specificity

Substrate (300mM)	Relative activity (%)
L-α-Glycerophosphate	100
Glucose-1-phosphate	0
Glucose-6-phosphate	0
Glycerol	0
Glucose	0

Table 2. Effect of various chemicals

Additive	Concentration	Relative activity (%)
None	2mM	100
$MgCl_2$	2mM	101
$MgSO_4$	2mM	102
$ZnCl_2$	2mM	102
$ZnSO_4$	2mM	102
NaCl	2mM	103
NH_4Cl	2mM	103
$BaCl_2$	2mM	103
$Ba(CH_3COO)_2$	2mM	101
$NiCl_2$	2mM	103
$CoCl_2$	2mM	103
$MnCl_2$	2mM	114
LiCl	2mM	103
KCl	2mM	102
$CaCl_2$	2mM	103
EMULGEN 810	0.1%	98
EMULGEN 911	0.1%	98
RHEODOL TWL-106	0.1%	99
RHEODOL 460	0.1%	99
ADEKANOL NP-720	0.1%	99
Triton X-100	0.1%	99
Triton X-305	0.1%	98
Tween 80	0.1%	100

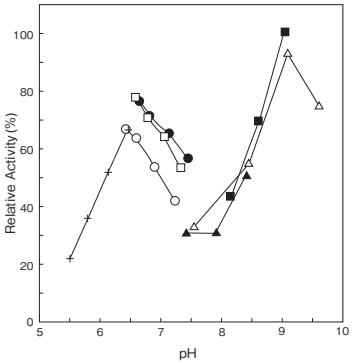
Table 3. GPOSP Turbidity test

Lot' Number \ Incubation Days	0	1	2	3	4	5	6	7
GPOSP not added	−	−	−	−	−	−	−	−
GPOSP (Lot 1)	−	−	−	−	−	−	−	−
GPOSP (Lot 2)	−	−	−	−	−	−	−	−
GPOS (Lot A)	−	−	±	±	++	++	++	++
GPOS (Lot B) existing product	−	±	±	+	++	++	++	++

− : Clear ± : Slight turbidity + : Milky ++ : Precipitation

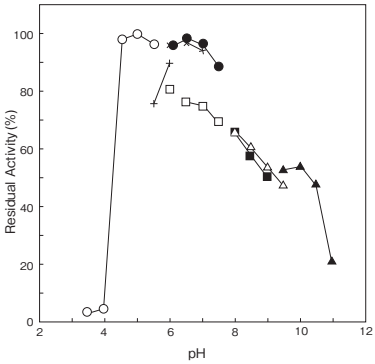
Storage conditions: 100 U/ml GPOSP or GPOS
50 mM PIPES pH 6.5, 0.05% NaN_3, 37℃

Fig.1 pH Optimum



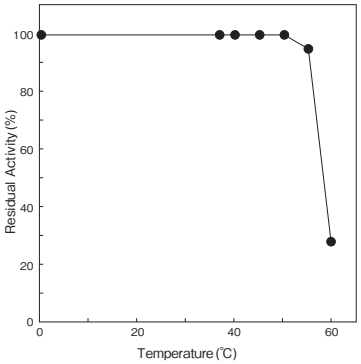
200 mM buffer
○ : MES buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : TEA buffer
+ : Citrate buffer

Fig.2 pH Stability



37℃, 30 min.
200 mM buffer
○ : Citrate buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : Glycine buffer
+ : MES buffer
× : Bis-Tris buffer

Fig.3 Thermal Stability



pH6.5, 5 min
100mM Phosphate buffer

Fig.4 Electrophoresis GPOSP

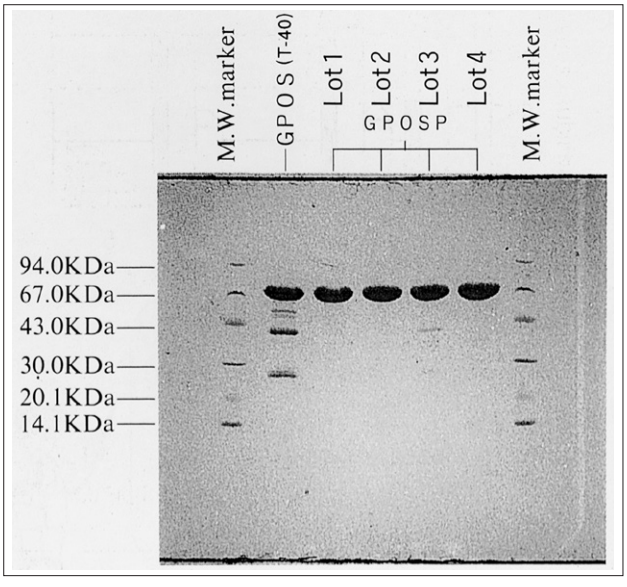
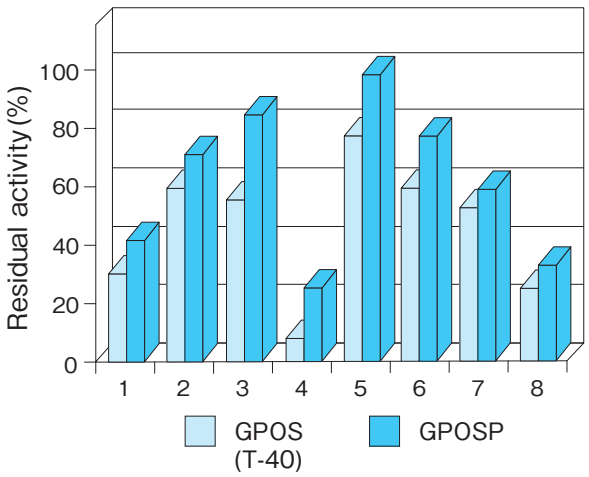


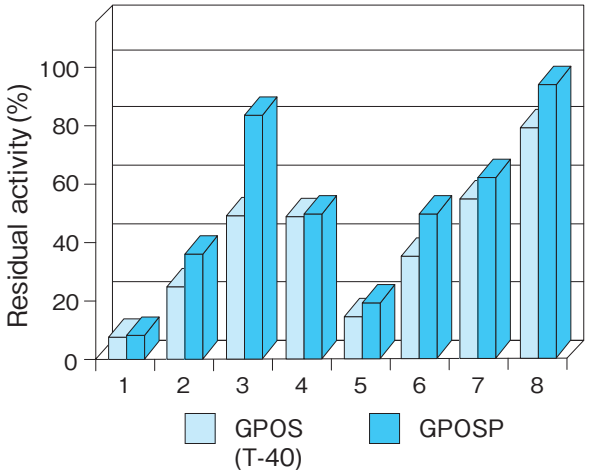
Fig.5 Liquid stability of GPOSP (Buffer, pH)



1 : MES pH 6.5
2 : Bis-Tris pH 6.0
3 : Bis-Tris pH 6.5
4 : Bis-Tris pH 7.0
5 : PIPES pH 6.0
6 : PIPES pH 6.5
7 : PIPES pH 7.0
8 : MOSPO pH 7.0

Incubation conditions : 37℃ for 7 days

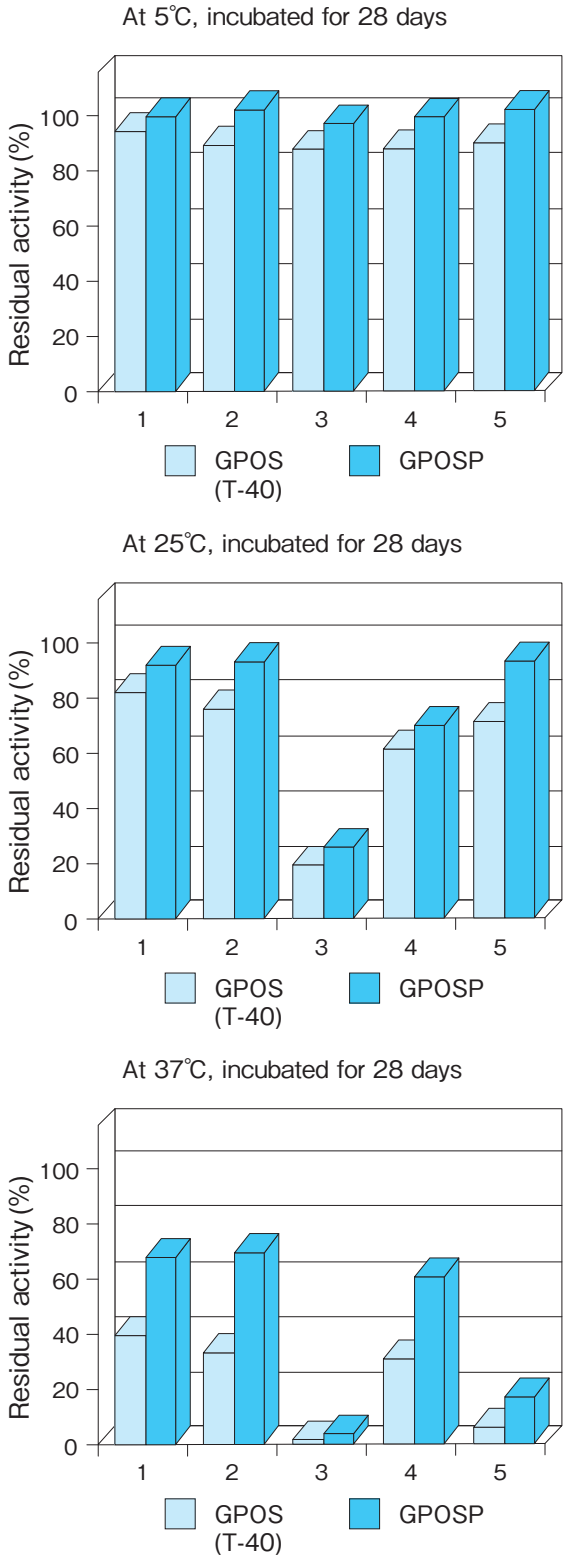
Fig.6 Liquid stability of GPOSP (Influence of detergents)



1 : EMULGEN 709
2 : EMULGEN B-66
3 : RHEODOL TWL-106
4 : RHEODOL 460
5 : Adekatol SO-120
6 : Adekatol B-795
7 : Triton X-100
8 : Triton X-305

Incubation conditions : 37℃ for 7 days

Fig.7 Antiseptic stability of GPOSP



1 : NaN_3 (0.03%)
2 : Kathon CG (0.01%)
3 : Propanol (0.02%)
4 : Kanamycin (0.05%)
5 : p-Hydroxymethylbenzoate (0.1%)

in 50 mM PIPES pH 6.5

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Assay

■ Principle

The assay is based on the increase in absorbance at 600 nm as the formation of quinoneimine dye in the following reactions:

$$\text{L-}\alpha\text{-Glycerophosphate} + O_2 \xrightarrow{\text{GPOSP}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

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

DAOS: [3, 5-dimethoxy-N-ethyl-N- (2-hydroxy-3-sulphopropyl) aniline]

■ Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of H_2O_2 per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture

   Dissolve 6.05g of PIPES and 9.45 g (purity calculation) of Disodium Glycerophosphate with 70 ml of distilled water and adjust pH to 6.5 with 4 N NaOH at 25℃. Add all reagents listed below and confirm pH is 6.5 at 25℃. Add distilled water to make a total of 100 ml.

100 U/ml POD [1] solution	5.0 ml
15 mM 4-AA solution	10.0 ml
100 mM DAOS solution	1.0 ml
5% (W/V) Triton X-100 solution	1.0 ml

   1): 100 U/ml POD solution

   Dissolve 1,000 U (PPU) of POD with 10 ml of distilled water.

2. Reaction stopper

   0.5% (W/V) SDS solution

   SDS: Sodium dodecyl sulfate

3. Enzyme dilution buffer

   10 mM PIPES－NaOH buffer pH 6.5

4. Reagents

   PIPES[Piperazine-1,4-bis (2-ethanesulfonic acid)]: Dojindo Laboratories #345-02225

   DAOS (sodium salt) : Dojindo Laboratories #OC06

   4-AA: NACALAI TESQUE, INC. Special grade #01907-52

   Triton X-100: The Dow Chemical Company

   Disodium Glycerophosphate 5.5 Hydrate : FUJIFILM Wako Pure Chemical Corporation #192-02055

   SDS (Sodium Dodecyl Sulfate) : NACALAI TESQUE, INC. Extra pure #31606-75

   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 1.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 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 5 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 600 nm.

   Absorbance sample : As

   blank : Ab

   0.050 Abs ≦ △A (As－Ab) ≦ 0.250 Abs

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/5}{16.8 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

16.8 : millimolar extinction coefficient of quinoneimine dye at 600 nm ($cm^2/\mu mole$)

1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye

5 : reaction time (min)

3.02 : 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. Enzyme activity will be retained for at least one year under this condition.

References

1. Jacobs, N. J. and Van Demark, P. J. (1960) Arch. Biochem. Biophys., **88**, 250-255.
2. Koditschek, L. K. and Umbreit, W. W. (1969) J. Bacteriol., **98**, 1063-1068.
3. Gancedo, C., Gancedo, J. M. and Sols, A. (1968) J. Biochem., **5**, 165-172.
4. Kistler, W. S., Hirsch, C. A., Cozzarelli, N. R. and Lin, E. C. C. (1969) J. Bacteriol., **100**, 1133-1135.
5. Esders, T. W. and Michrina, C. A. (1979) J. Biol. Chem., **254**, 2710-2715.

GPOSP 活性測定法（Japanese）

Ⅰ．試薬液

1. 反応試薬混合液

PIPES 6.05g とグリセロりん酸 2Na 9.45g（純度換算）を精製水 70ml に溶解した後、4N NaOH で pH6.5（25℃）に調整し、その液に下記試薬を加えて混和し、pH6.5（25℃）であることを確認した後、精製水で全容 100ml とする。

100U/ml POD 溶液 [1)]	5.0 ml
15mM 4–AA 溶液	10.0 ml
100mM DAOS 溶液	1.0 ml
5%（W/V）トリトン X–100 溶液	1.0 ml

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

2. 反応停止液
0.5%（W/V）SDS 溶液

3. 酵素溶解希釈用液
10mM PIPES–NaOH 緩衝液 pH6.5

4. 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
DAOS［3,5– ジメトキシ –N– エチル –N–（2– ヒドロキシ –3– スルフォプロピル）アニリン］:
同仁化学製　#OC06
4–AA: ナカライテスク製　特級　#01907–52
トリトン X–100: Dow Chemical 製
グリセロりん酸二ナトリウム 5.5 水和物:
富士フイルム和光純薬製　#192–02055
SDS（ドデシル硫酸ナトリウム）:
ナカライテスク製　一級　#31606–75
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 1.0ml 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 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わりに酵素溶解希釈用液 20 μl を加える。
3. 5 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. 600nm 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。

$$0.050\ \text{Abs} \leqq \Delta A = (As - Ab) \leqq 0.250\ \text{Abs}$$

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5}{16.8 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

16.8 : キノンイミン色素の 600nm におけるミリモル分子吸光係数（$cm^2/\ \mu mole$）
1/2 : H_2O_2 2 モルからキノン色素 1 モルが生成することによる係数
5 : 反応時間（min）
3.02 : 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）