

(Diagnostic Reagent Grade)

T-60

L- α -GLYCEROPHOSPHATE OXIDASE [GPOSP]

from *Streptococcus* sp.

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


★ Advantages

- ① Highly purified enzyme
- ② Stability in solution
- ③ Resistance 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

pH stability : 5.0-7.0 (37°C, 30 min)

Optimum temperature : 37°C

Thermal stability : Stable at 55°C and below
(100 mM Phosphate buffer pH 6.5, 5 min.)

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

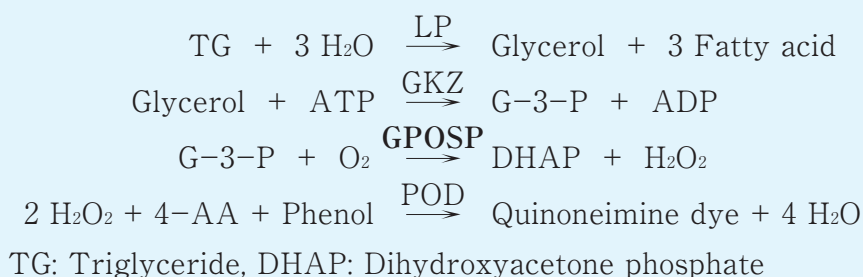
This enzyme is useful for enzymatic determination of **triglyceride**.


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 3. GPOSP Turbidity test

Incubation Days	0	1	2	3	4	5	6	7
Lot' Number								
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% NaN₃, 37°C

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 ₄ Cl	2mM	103
BaCl ₂	2mM	103
Ba(CH ₃ COO) 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

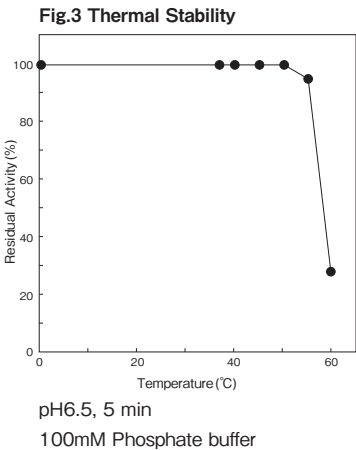
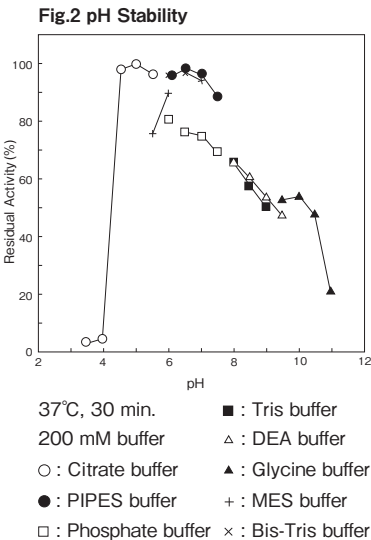
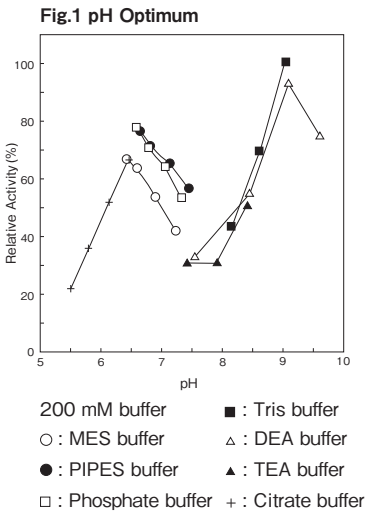


Fig.4 Electrophoresis GPOSP

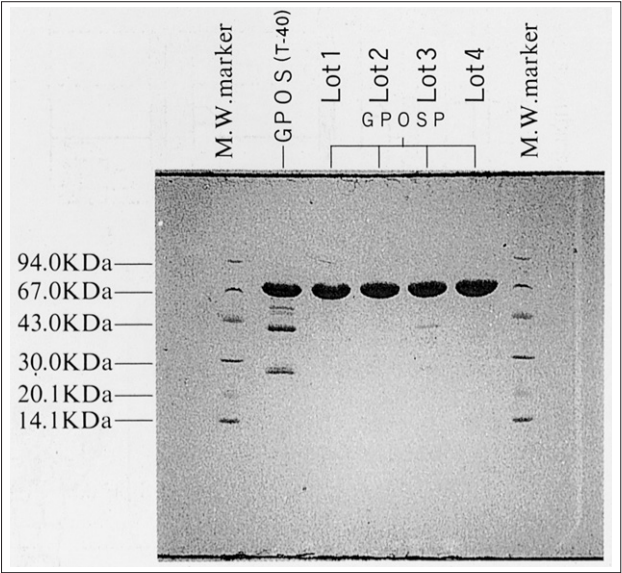


Fig.5 Liquid stability of GPOSP(Buffer, pH)

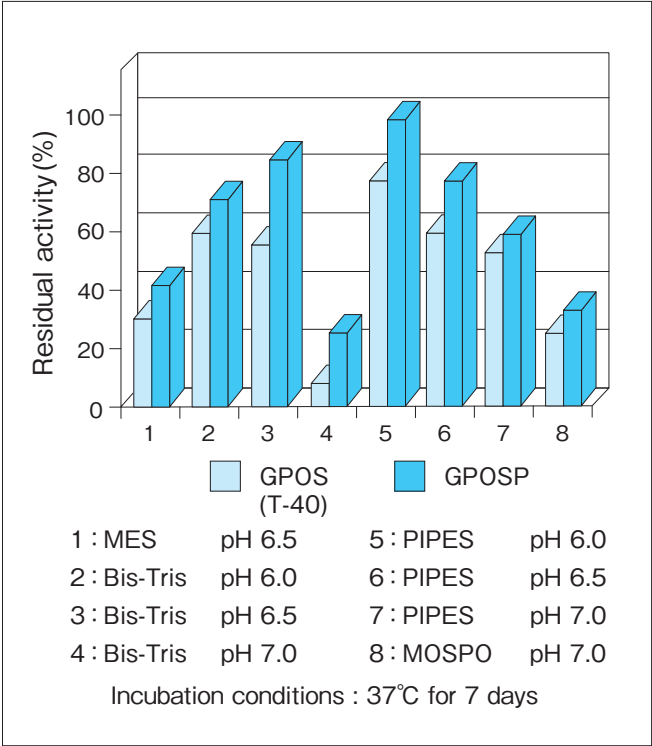


Fig.6 Liquid stability of GPOSP (Influence of detergents)

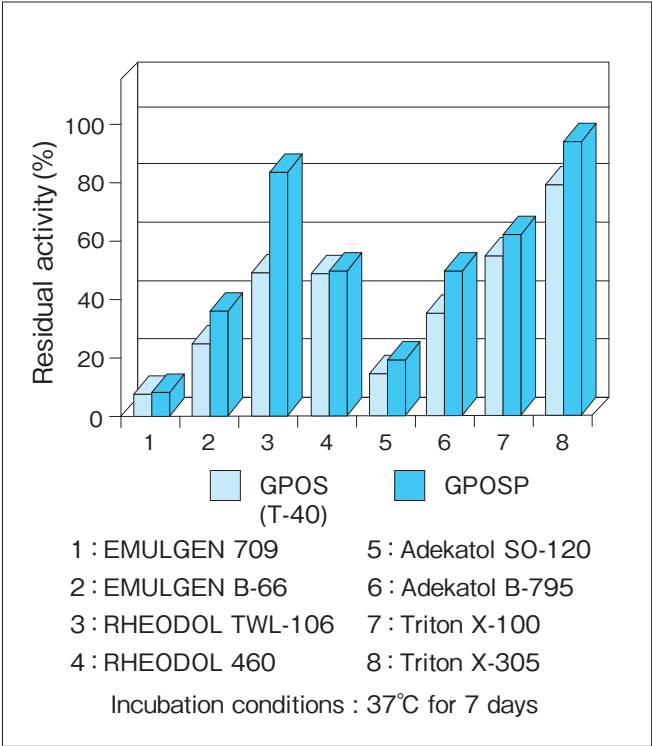
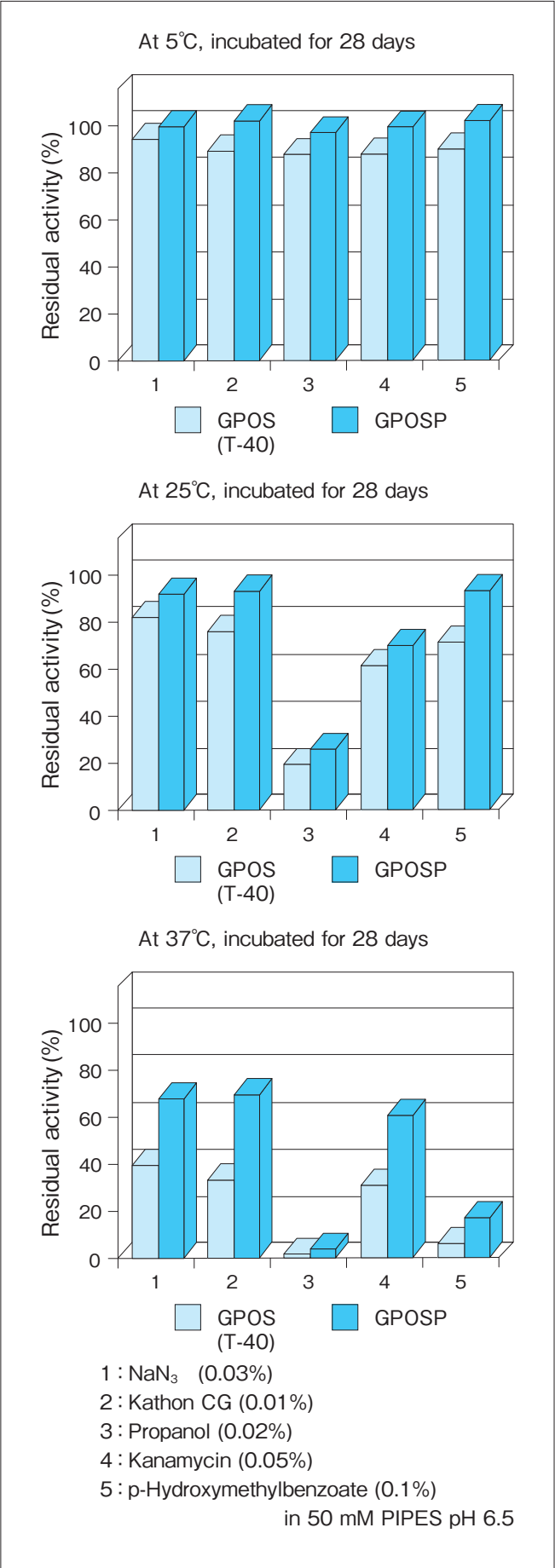


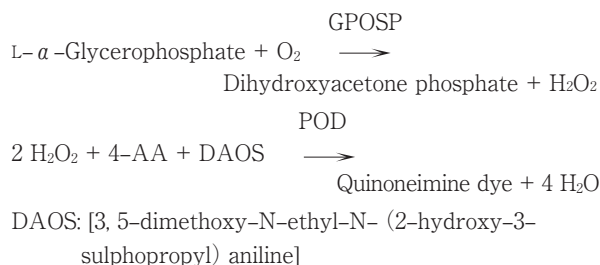
Fig.7 Antiseptic stability of GPOSP



Assay

■ Principle

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



■ Unit definition

One unit is defined as the amount of enzyme which generates 1 μmole of H_2O_2 per minute at 37°C 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°C . Add all reagents listed below and confirm pH is 6.5 at 25°C . 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°C .

2. After 5 min, add exactly 20 μl of enzyme solution and mix to start the reaction at 37°C .

※ 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

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

■ Calculation

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

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

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°C 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 とグリセロリン酸 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

グリセロリン酸二ナトリウム 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 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わりに酵素溶解希釈用液 20 μ l 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。

$$0.050 \text{ Abs} \leq \Delta A = (A_s - A_b) \leq 0.250 \text{ Abs}$$

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 ($\text{cm}^2 / \mu\text{mole}$)

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)