L-α-GLYCEROPHOSPHATE OXIDASE [GPOM]

from *Streptococcus* sp.
(sn-Glycerol-3-phosphate: oxygen 2-oxidoreductase, EC 1. 1. 3. 21)

L-α-Glycerophosphate + O_2 ⟶ Dihydroxyacetonephosphate + H_2O_2

Preparation and Specification

Appearance : Yellowish amorphous powder, lyophilized
Specific activity : More than 15 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 169 kDa (TSK gel G 3000 SW$_{XL}$ gel filtration) 65 kDa (SDS-PAGE)	
Isoelectric point	: pH 4.4	
Michaelis constants	: L-α-Glycerophosphate 0.64 mM (pH 7.5)	
Optimum pH	: 8.5-9.0	Figure 1
pH stability	: 6.0-8.0 (37℃, 30 min)	Figure 2
Optimum temperature	: 37-42℃ (pH 6.5)	Figure 3
Thermal stability	: Stable at 40℃ and below (pH 6.5)	Figure 4
Effect of metal ions	: See Table 2	
Effect of detergents	: See Table 3	
Stabilizers	: FAD	

Applications for Diagnostic Test

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

$$\text{TG} + 3\,H_2O \xrightarrow{\text{LP}} \text{Glycerol} + 3\ \text{Fatty acid}$$
$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GKZ}} \text{G-3-P} + \text{ADP}$$
$$\text{G-3-P} + O_2 \xrightarrow{\textbf{GPOM}} \text{DHAP} + H_2O_2$$
$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

TG: Triglyceride, DHAP: Dihydroxyacetonephosphate

Table 1. Substrate specificity

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

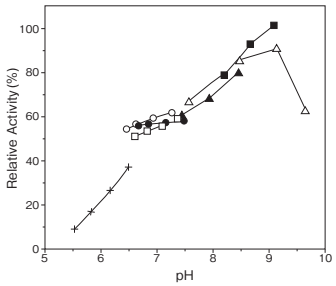
Table 2. Effect of metal ion on GPOM activity

Metal ion (2mM)	Relative activity (%)
None	100
$MgCl_2$	101
$MgSO_4$	102
$ZnCl_2$	102
$ZnSO_4$	102
NaCl	103
NH_4Cl	103
$BaCl_2$	103
$Ba(CH_3COO)_2$	101
$NiCl_2$	103
$CoCl_2$	103
$MnCl_2$	114
LiCl	103
KCl	102
$CaCl_2$	103

Table 3. Effect of detergents on GPOM activity

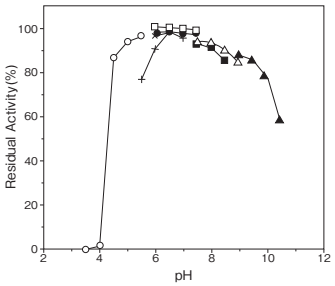
Detergent (0.1%)	Relative activity (%)
None	100
EMULGEN 810	98
EMULGEN 911	98
RHEODOL TWL-106	99
RHEODOL 460	99
ADEKANOL NP-720	99
Triton X-100	98
Triton X-305	99
Tween 80	98

Fig.1 Optimum pH



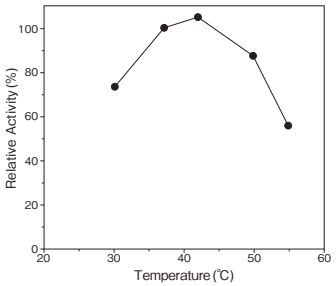
200 mM buffer
○ : MES buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : TEA buffer
+ : Citrate buffer

Fig.2 pH Stability



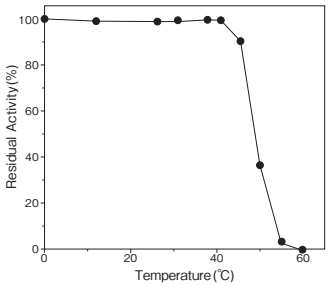
37℃, 30 min.
200 mM buffer
○ : Citrate buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : Glycine buffer
+ : MES buffer
× : Bis Tris buffer

Fig.3 Optimum Temperature



pH 6.5
200 mM PIPES buffer

Fig.4 Thermal Stability



pH 6.5, 10min.
50 mM PIPES buffer

Assay

■ Principle

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

$$\text{L-}\alpha\text{-Glycerophosphate} + O_2 \xrightarrow{\text{GPOM}} \text{Dihydroxyacetonephosphate} + H_2O_2$$

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

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 6.05 g 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

100mM 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
containing 0.1% (W/V) Triton X-100

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

0.1 Abs ≦ △A (As − Ab) ≦ 0.2 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 / μmole)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces l 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. (Tokyo), **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.

GPOM 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
0.1% (W/V) トリトン X-100 を含む 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。

$$0.1\ \mathrm{Abs} \leqq \Delta A = (As - Ab) \leqq 0.2\ \mathrm{Abs}$$

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)