(Diagnostic Reagent Grade) ASAHI KASEI ENZYMES T−107

L−α−GLYCEROPHOSPHATE OXIDASE [GPOM]

from *Streptococcus* sp., genetically engineered recombinant
(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 SWXL 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	

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

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

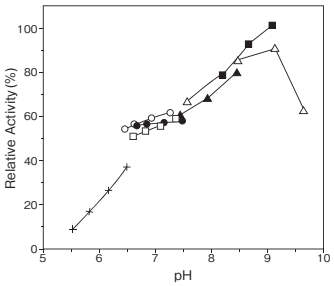
Table 2. Effect of metal ion on GPOM activity

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

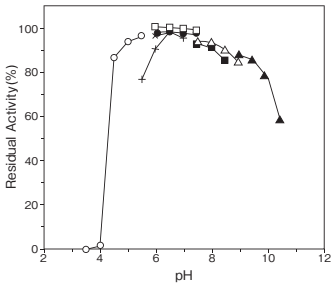
Detergent (0.1%)	Relative activity (%)
None	100
EMULGEN 810	98
EMULGEN 911	98
RHEODOL TWL-106	99
RHEODOL 460	99
ADEKANOL NP-720	99
Triton X-100	98
Triton X-305	99
Tween 80	98

Fig.1 Optimum pH



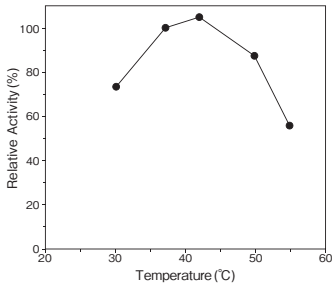
200 mM buffer
○ : MES buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : TEA buffer
+ : Citrate buffer

Fig.2 pH Stability



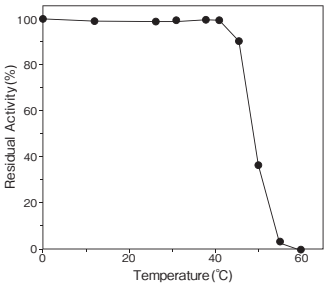
37℃, 30 min.
200 mM buffer
○ : Citrate buffer
● : PIPES buffer
□ : Phosphate buffer
■ : Tris buffer
△ : DEA buffer
▲ : Glycine buffer
+ : MES buffer
× : Bis Tris buffer

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 qui noneimine 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 9.72 g (purity calculation) of DL-α-glycerophosphate (G-1-P) 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 [2] -NaOH buffer pH 6.5
containing 0.1% (W/V) Triton X-100
2):PIPES: [Piperazine-N, N'-bis (2-ethanesulfonic acid)]

4. Reagents
PIPES: 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
G-1-P (DL-α-glycerophosphate·2Na):
Sigma Chemical Co. #G-2138
SDS: NACALAI TESQUE, INC.
Extra pure #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 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 (Figure 4).

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 G-1-P 9.72g (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%Triton X-100 を含む 10mM PIPES-NaOH 緩衝液 pH6.5

4. 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
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 : ナカライテスク社製 特級 #01907-52
トリトン X-100 : Dow Chemical 社製
G-1-P (DL-グリセロ-3-リン酸·2Na):
シグマ社製 X #G-2138
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 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)