

# PYRUVATE OXIDASE [POPG]

from *Aerococcus viridans*

(Pyruvate: oxygen oxidoreductase, phosphorylating, EC 1.2.3.3)



## Preparation and Specification

Appearance : Yellowish amorphous powder, lyophilized

Specific activity : More than 25 U/mg solid

Contaminants :

Lactate oxidase	Less than 0.002 % (U/U)
Total AST (GOT)	Less than 0.002 % (U/U)
Total ALT (GPT)	Less than 0.006 % (U/U)
Catalase	Less than 0.3 % (U/U)

## Properties

Substrate specificity : See Table 1

Molecular weight : 155 kDa (gel filtration)  
70 kDa (SDS-PAGE)

Isoelectric point : pH 4.0

Michaelis constants : Pyruvate  $5.9 \times 10^{-3}\text{M}$  ( $\text{Mn}^{2+}$ )  
Pyruvate  $4.1 \times 10^{-2}\text{M}$  ( $\text{Mg}^{2+}$ )  
Phosphate  $2.0 \times 10^{-3}\text{M}$  ( $\text{Mn}^{2+}$ )  
Phosphate  $4.5 \times 10^{-3}\text{M}$  ( $\text{Mg}^{2+}$ )

Optimum pH : 6.5–7.0

pH stability : 6.0–7.0 (37°C, 60 min, 10  $\mu$  M FAD)

Optimum temperature : 40°C

Thermal stability : Stable at 45°C and below (phosphate buffer  
containing 10  $\mu$  M FAD, pH 6.5, 10 min)

Storage stability : At least one year at -20°C

Stabilizer : FAD

Activators :  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$

Inhibitors : EDTA

Co-factors : FAD, TPP

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

## Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **AST and ALT** when coupled with oxaloacetate decarboxylase (T-14) (in the case of AST) and POD.

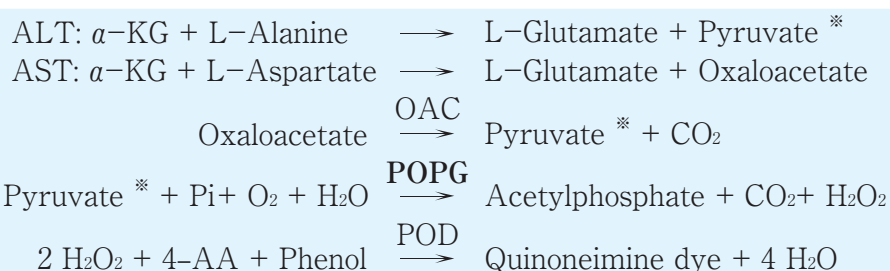
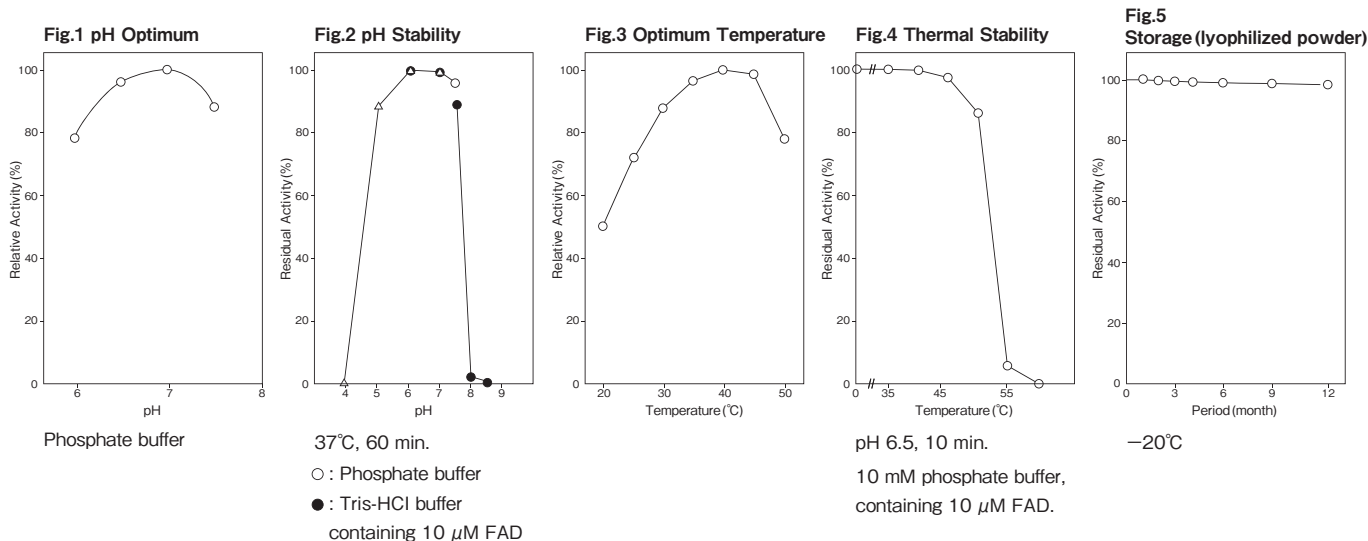


Table 1. Substrate specificity

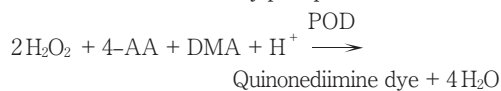
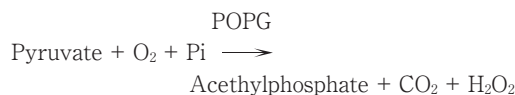
Substrate	Relative activity (%)
Pyruvate	100
2-Oxobutyrate	3
2-Oxoglutarate	0
Oxaloacetate	0
DL-Lactate	0
Acetate	0
L-Alanine	0
L-Aspartate	0



## Assay

### Principle

The assay is based on the increase in absorbance at 565 nm as the formation of quinoneimine dye proceeds in the following reactions:



DMA: N, N-Dimethylaniline

### Unit definition

One unit is defined as the amount of enzyme which generates 1 μ mole of H<sub>2</sub>O<sub>2</sub> per minute at 37°C under the conditions specified in the assay procedure.

### Reagents

- Reaction mixture I
 

1 M KH <sub>2</sub> PO <sub>4</sub> -NaOH buffer pH 6.7	0.20 ml
10 mM Thiamine pyrophosphate	0.02 ml
1 mM FAD solution	0.01 ml
100 U/ml POD solution <sup>1)</sup>	0.05 ml
15 mM 4-AA solution	0.10 ml
Distilled water	0.22 ml

FAD: Flavine adenine dinucleotide  
 1): 100 U/ml POD solution  
 Dissolve 1,000 U (PPU) of POD with 10 ml of distilled water.

- Reaction mixture II
 

0.2 % (V/V) DMA solution	0.20 ml
0.1 M MgCl <sub>2</sub> solution	0.10 ml
- Substrate solution  
1 M Potassium pyruvate solution
- Reaction stopper  
McIlvaine buffer pH 5.50-5.55 containing 0.1 M EDTA  
EDTA: Ethylenediamine tetraacetic acid
- Enzyme dilution buffer  
10 mM KH<sub>2</sub>PO<sub>4</sub>-NaOH buffer pH 7.0 containing 10 μM FAD
- Reagents  
Thiamine pyrophosphate (Cocarboxylase):  
Wako Pure Chemical Industries, Ltd. #031-03833  
4-AA: NACALAI TESQUE, INC. Special grade #01907-52  
POD: Sigma Chemical Co. Type II #P-8250  
DMA (2Na): Wako Pure Chemical Industries, Ltd.  
Special grade #044-02763  
FAD (2Na): Kyowa Hakko Co., Ltd.  
Potassium pyruvate:  
Wako Pure Chemical Industries, Ltd.  
(for biochemistry) #166-08351  
EDTA (2Na·2H<sub>2</sub>O): KISHIDA CHEMICAL Co., Ltd.  
#060-29133

### 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 solution buffer to adjust the concentration as required.

## ■ Procedure

- Mix reaction mixture I, substrate solution and reaction mixture II in a ratio of 6 : 1 : 3. Pipette accurately 1.0 ml of the mixture into a small test tube and preincubate at 37°C.
- After 5 min, add exactly 20  $\mu$ l of enzyme solution and mix to start the reaction at 37°C.  
※ In the case of a test blank, add 20  $\mu$ l of enzyme dilution buffer in place of enzyme solution.
- At 10 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
- After 5 min, measure the absorbance at 565 nm.

$$\begin{aligned} \text{Absorbance sample} &: \text{As} \\ \text{blank} &: \text{Ab} \\ \Delta A &= (\text{As} - \text{Ab}) \leq 0.400 \text{ Abs} \end{aligned}$$

## ■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\Delta A / 10}{23.56 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

23.56 : millimolar extinction coefficient of quinonodimine dye at 565 nm ( $\text{cm}^2 / \mu\text{mole}$ )

1/2 : a multiplier derived from the fact that 2 mole of

$\text{H}_2\text{O}_2$  produces 1 mole of quinoneimine dye

- 10 : 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  $-20^\circ\text{C}$  in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition (Figure 5).

## References

- Harger, L. P., Geller, D. M. and Lipmann, F. (1954) Fed. Proc., **13**, 734-738.
- Lipmann, F. (1940) J. Biol. Chem., **134**, 463-464.
- Harger, L. P. and Lipmann, F. (1955) Methods Enzymol., Vol. **1**, 482.
- Sedewitz, B., Schleifer, K. H. and Gotz, F. (1984) J. Bacteriol., **160**, 273-278.
- Sedewitz, B., Schleifer, K. H. and Gotz, F. (1984) J. Bacteriol., **160**, 462-465.

## POPG 活性測定法 (Japanese)

### I. 試薬液

- 反応試薬混合液 I
 

1M $\text{KH}_2\text{PO}_4$ - NaOH 緩衝液 pH6.7	0.20 ml
精製水	0.22 ml
15mM 4-AA 溶液	0.10 ml
100U/ml POD 溶液 <sup>1)</sup>	0.05 ml
10mM チアミンピロリン酸	0.02 ml
1mM FAD 溶液	0.01 ml

1): 100U/ml POD 溶液  
POD 1,000 単位 (PPU) を精製水 10ml で溶解する。
- 反応試薬混合液 II
 

0.2% (V/V) DMA 溶液	0.20 ml
0.1M 塩化マグネシウム溶液	0.10 ml
- 基質溶液
 

1M ピルビン酸カリウム溶液
- 反応停止液
 

0.1M EDTA を含むマックイルバイン緩衝液 pH5.50~5.55
- 酵素溶解希釈用液
 

10  $\mu$  M FAD を含む 10mM  $\text{KH}_2\text{PO}_4$  - NaOH 緩衝液 pH7.0
- 試薬
 

チアミンピロリン酸 (コカルボキシラーゼ):  
和光純薬工業製 特級 #031-03833  
POD: シグマ社製 Type II #P-8250  
4-AA: ナカライテスク社製 特級 #01907-52  
DMA (N,N'-ジメチルアニリン):  
和光純薬工業製 特級 #044-02763  
FAD (フラビンアデニンヌクレオチド $\cdot$ 2Na):  
協和発酵製

ピルビン酸カリウム:

和光純薬工業製 生化学用 #166-08351

EDTA (エチレンジアミン四酢酸 $\cdot$ 2Na $\cdot$ 2H $_2$ O):

キシダ化学社製 #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

- 反応試薬混合液 I と基質溶液及び反応試薬混合液 II を 6:1:3 に混合し、その混合液 1.0ml ずつを正確に小試験管へ分注して 37°C で予備加温する。
- 5 分経過後、酵素試料液 20  $\mu$ l を加えて混和し、37°C 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  $\mu$ l を加える。
- 10 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。
- 5 分後、565nm における吸光度を測定する。  
求められた吸光度変化の試料液は As、盲検液は Ab とする。

$$\Delta A = (\text{As} - \text{Ab}) \leq 0.400 \text{ Abs}$$

### IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A / 10}{23.56 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

23.56: キノンジイミン色素の 565nm におけるミリモル分子吸光係数 ( $\text{cm}^2 / \mu\text{mole}$ )

1/2:  $\text{H}_2\text{O}_2$  2 モルからキノンジイミン色素 1 モルが生成することによる係数

10 : 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)