# LACTATE OXIDASE [LOX Ⅱ]

from *Aerococcus viridans*
(L-Lactate: oxygen oxidoreductase, EC 1.1.3.2)

L-Lactate + $O_2$ ⟶ Pyruvate + $H_2O_2$

## Preparation and Specification

| | |
|---|---|
| Appearance | : Yellowish amorphous powder, lyophilized |
| Specific activity | : More than 20 U/mg solid |
| Contaminants | : |
| POP | Less than 0.001 % (U/U) |
| GOD | Less than 0.001 % (U/U) |
| UODN | Less than 0.001 % (U/U) |
| CO | Less than 0.001 % (U/U) |

## Properties

| | | |
|---|---|---|
| Molecular weight | : 80 kDa (gel filtration) | |
| Isoelectric point | : pH 4.6 | |
| Michaelis constant | : L-Lactate 7.0 × $10^{-4}$M | |
| Optimum pH | : 6.0–7.0 | Figure 1 |
| pH stability | : 6.0–9.0 (50℃, 10 min) | Figure 2 |
| Optimum temperature | : 35℃ | |
| Thermal stability | : Stable at 50℃ and below (pH 8.5, 10 min) | Figure 3 |
| Storage stability | : At least one year at −20℃ | Figure 4 |

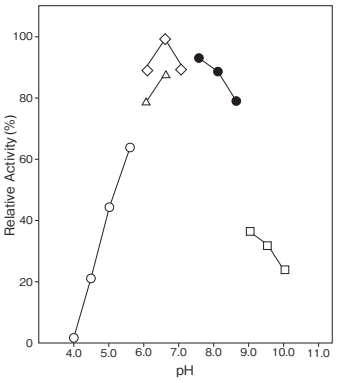
## Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **lactic acid**.

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOX Ⅱ}} \text{Pyruvate} + H_2O_2$$

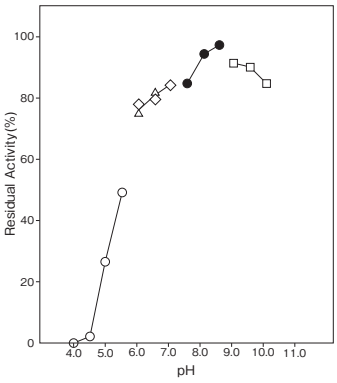
$$2\,H_2O_2 + \text{4-AA} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 4\,H_2O$$

**Fig.1 pH Optimum**



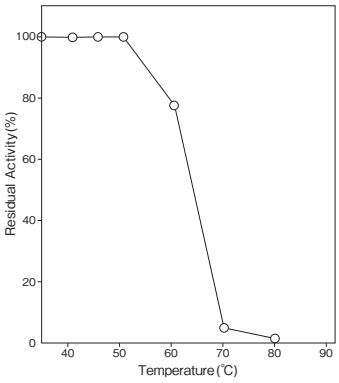

○ : Acetate buffer
△ : Dimethyl glutarate-NaOH buffer
◇ : $KH_2PO_4$-$K_2HPO_4$ buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer

**Fig.2 pH Stability**



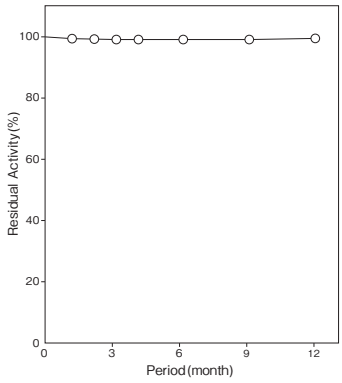

40 mM buffer, 50℃, 10 min.
○ : Acetate buffer
△ : Dimethylglutarate-NaOH buffer
◇ : $KH_2PO_4$-$K_2HPO_4$ buffer
● : Tris-HCl buffer
□ : Glycine-NaOH buffer

**Fig.3 Thermal Stability**




pH 8.5, 10 min.
40 mM Tris-HCl buffer

**Fig.4 Storage (lyophilized powder)**




−20℃

## 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:

$$\text{L-Lactate} + O_2 \xrightarrow{\text{LOX II}} \text{Pyruvate} + H_2O_2$$

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

DMA: N, N'-Dimethylaniline

### ■ 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

| | |
|---|---|
| 0.2 M $KH_2PO_4$-NaOH buffer pH 6.5 | 0.2 ml |
| 50 U/ml POD solution [1)] | 0.1 ml |
| 15 mM 4-AA solution | 0.1 ml |
| 0.5 M DL-Lactic acid solution pH 6.5 | 0.1 ml |
| Distilled water | 0.3 ml |

Mix above reagents in advance. Just before measuring, add the reagent listed below and mix.

| | |
|---|---|
| 0.2% (W/V) DMA solution | 0.2 ml |

1): 50 U/ml POD solution
Dissolve 500 U (PPU) of POD with 10 ml of distilled water.

2. Reaction stopper
0.25% (W/V) LBS solution
LBS: Sodium lauryl sulfate
3. Enzyme dilution buffer
10 mM $KH_2PO_4$-NaOH buffer pH 7.0 containing 10 μM FAD
FAD: Flavine adenine dinucleotide
4. Reagents
DL-Lactic acid:
FUJIFILM Wako Pure Chemical Corporation
Special grade #128-00056
DMA: FUJIFILM Wako Pure Chemical Corporation
Special grade #044-02763
FAD (2Na): Kyowa Hakko Co., Ltd.
LBS: NACALAI TESQUE, INC. Extra pure #20123-22
4-AA: NACALAI TESQUE, INC.
Special grade #01907-52
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 solution 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 10 min after starting the reaction, add 2.0 ml of the reaction stopper to stop the reaction.
4. Measure the absorbance at 565 nm.

Absorbance sample : As
blank : Ab
$\triangle A = (As - Ab) \leqq 0.350$ Abs

### ■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/10}{35.33 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

35.33 : millimolar extinction coefficient of quinoneimine dye at 565 nm ($cm^2$ / μmole)
1/2 : a multiplier derived from the fact that 2 mole of

$H_2O_2$ 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℃ in the presence of a desiccant is recommended. The enzyme activity will be retained for at least one year under this condition (Figure 4).

## References

1. Eichel, H. J. and Rem, L. T. (1962) J. Biol. Chem., **237**, 940–945.
2. Esders, T. W. and Goodhue, C. T. (1980) Eastman Kodak Company, U. S. Pat. 4,241,178.

## LOX Ⅱ活性測定法 (Japanese)

**Ⅰ. 試薬液**

1. 反応試薬混合液

| | |
|---|---|
| 0.2M $KH_2PO_4$–NaOH 緩衝液 pH6.5 | 0.2 ml |
| 50U/ml POD 溶液 [1)] | 0.1 ml |
| 15mM 4–AA 溶液 | 0.1 ml |
| 0.5M DL–乳酸溶液 pH6.5 | 0.1 ml |
| 精製水 | 0.3 ml |

を混合して置く。測定直前に前溶液と
0.2% (V/V) DMA 溶液 0.2 ml
を混合する。

1): 50U/ml POD 溶液
POD 500 単位 (PPU) を精製水 10ml で溶解する。

2. 反応停止液
0.25% (W/V) LBS 溶液
3. 酵素溶解希釈用液
10 μM FAD を含む 10mM $KH_2PO_4$–NaOH 緩衝液 pH7.0
4. 試薬
POD:シグマ製 Type Ⅱ #P–8250
4–AA:ナカライテスク製 特級 #01907–52
乳酸 (DL–Lactic acid):
富士フイルム和光純薬製 特級 #128–00056
DMA (N,N'–ジメチルアニリン):
富士フイルム和光純薬製 特級 #044–02763
FAD (フラビンアデニンジヌクレオチド·2Na):
協和発酵製
LBS (ラウリルベンゼンスルホン酸ナトリウム):
ナカライテスク製 Extra pure #20123–22

**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 を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 20 μl を加えて混和し、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. 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。
4. 565nm 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。

$$\Delta A = (As - Ab) \leqq 0.350\ Abs$$

**Ⅳ. 計算**

$$\text{活性 (U/mg)} = \frac{\Delta A/10}{35.33 \times 1/2} \times \frac{3.02}{0.02} \times \frac{1}{X}$$

35.33 : キノンイミン色素の 565nm におけるミリモル分子吸光係数 ($cm^2$/ μmole)
1/2 : $H_2O_2$ 2 モルからキノンイミン色素 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)