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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Lactate Oxidase (LOX)

Cat no. LDG0036RG

Product Overview

Description

Lactate oxidase (LOX) is an enzyme that catalyzes the oxidation of L-lactate to pyruvate and hydrogen peroxide. This flavoprotein, primarily found in bacteria and fungi, plays a crucial role in various biological processes, including energy metabolism and redox regulation. Due to its high specificity for lactate, LOX is widely utilized in biosensors for detecting lactate in clinical diagnostics, food quality control, and sports science. Its application allows for accurate and rapid quantification of lactate levels.

Expression system

Escherichia coli

Specification

Appearance	Yellowish amorphous powder, lyophilized
Activity	200 U/mg or more

Properties

Stability	Stable at -20℃ for at least six months
Isoelectric point	5.45

Reconstitution

It is recommended to weight and reconstitute 10 mg of lyophilized powder in 200 µL double-distilled water directly (final activity is 10 U/ µL) and incubate the solution for at least 10 mins to ensure sufficient re-dissolved.

Applications

1. Enzymatic determination of L-Lactate
2. Biosensor development [1]
3. Lactate detection in food industry [1]
4. Detection of lactate concentration in blood as a diagnostic parameter [1]

Assay

1. Assay principle

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

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{EHSPT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O$$

2. Unit definition

One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the following conditions:
20 mM potassium phosphate buffer (pH 7.5), 48 mM D-L-lactate, 1.2 mM 4-aminoantipyrine, 0.76 mM EHSPT, and 2.4 U/mL peroxidase.

3. Reagent

A. DL-Lactate solution	0.125 M [120 mg of DL-lithium lactate (MW=96.01)/10 mL of 50 mM K-Phosphate buffer pH 7.5] (Should be prepared fresh)

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B. 4-AA solution	0.5% (500 mg of 4-aminoantipyrine/100 mL of H_2O) (Store at 4°C in a brownish bottle)
C. EHSPT (TOOS) solution	20 mM [296 mg N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine(MW=295.3)/50 mL of H_2O] (Store at 4℃ in a brownish bottle)
D. Peroxidase solution	25 U/mL [Prepare a stock containing 20 mg of horseradish peroxidase (300 units/mg) /2 mL of H_2O, and dilute the stock to 25 U/mL]
E. SDS solution	0.25% (500 mg sodium dodecyl sulfate/200 mL of H_2O)
F. Enzyme diluent	20 mM K-Phosphate buffer, pH 7.0 containing 0.1% (w/v) sodium cholate

4. Procedure

(1) Prepare the following **Working Solution** immediately before use and equilibrate at 37℃ for approximately 5 minutes (for 8 reactions).

Working Solution

DL-Lactate solution (Reagent A)	1.6 mL
4-AA solution (Reagent B)	0.24 mL
EHSPT solution (Reagent C)	0.16 mL
Peroxidase solution (Reagent D)	0.4 mL
Distilled water	1.6 mL
Total	**4 mL**

(2) Pipette 0.5 mL of **Working Solution** into a tube.

(3) Add 0.025 mL of the enzyme solution* and mix with a gentle inversion.

Concentration in a reaction	
K-phosphate buffer	20 mM
DL-Lactate	48 mM
4-Aminoantipyrine	1.2 mM
EHSPT	0.76 mM
Peroxidase	2.4 U/mL

(4) After exactly 15 minutes at 37°C, add 1 mL of SDS solution **(Reagent E)** to stop the reaction and measure the optical density at 555 nm against water (OD test).

(5) At the same time, prepare the blank by using the same method as the test except that the Enzyme diluent **(Reagent F)** is used instead of the enzyme solution (OD blank).

* Dissolve the enzyme preparation in ice-cold Enzyme diluent **(Reagent F)** dilute to **0.04–0.1 U/mL** with the same buffer and store on ice.

(6) Activity can be calculated by using the following formula:

Volume activity (U/mL) =

$$\frac{\Delta OD\ (OD\ test - OD\ blank) \times Vt \times df}{34.3 \times 1/2 \times t \times 1.0 \times Vs}$$

$$= \Delta OD \times 0.237 \times df$$

Weight activity (U/ mg) = (U/mL) × 1/C

Vt: Total volume (1.525 mL)

Vs: Sample volume (0.025 mL)

34.3: Millimolar extinction coefficient of quinoneimine dye under the assay condition (cm^2/micromole)

1/2: The factor is derived from the stoichiometric relationship in which one mole of H_2O_2 yields half a mole of quinoneimine dye.

t: Reaction time (15 minutes)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/mL)

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Reference

1. *Agustina Godino, et al.* His-tagged lactate oxidase production for industrial applications using fed-batch fermentation. *Journal of Biotechnology* (2023).

Disclaimer

For Research Use or Further Manufacturing Only.

The effect of different conditions on Lactate Oxidase

A.

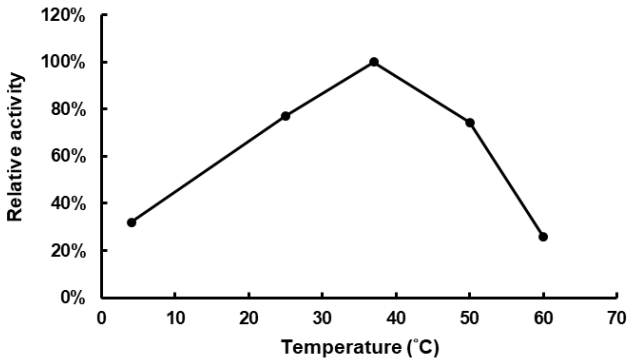

Figure A. Temperature activity of LOX. The enzyme reactions in 20 mM K-Phosphate buffer, pH 7.5, were carried out under different temperatures.

B.

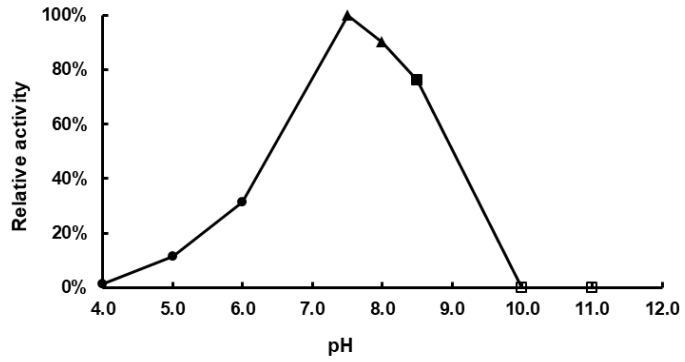

Figure B. pH activity of LOX. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.5-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

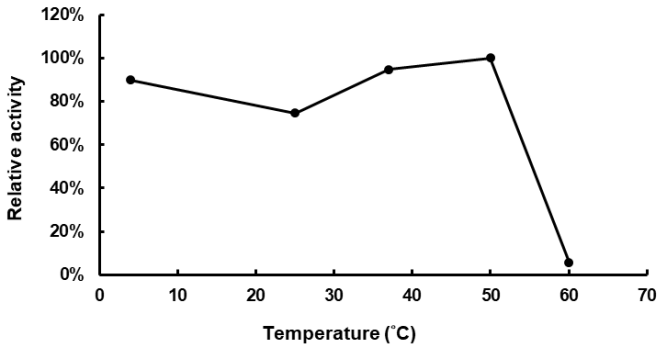

Figure C. Thermal stability of LOX. The enzyme powder was reconstituted by double-distilled water and treated at different temperatures for 10 minutes. Final concentration: 10 U/mL.

D.

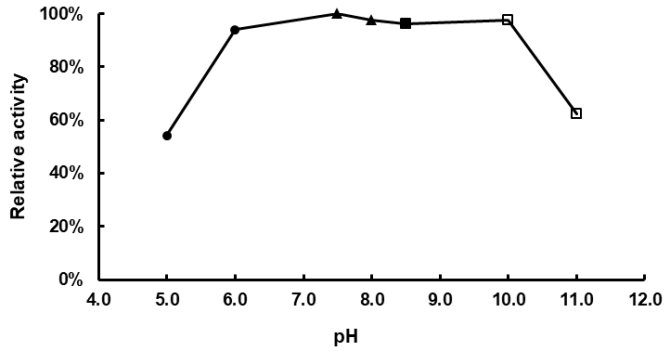

Figure D. pH stability of LOX. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition at 25°C for 16 hours. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.5-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

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