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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Lactate Oxidase (LOX)

Cat no. LDG0033RG

Product Overview

Specification

Appearance	Yellowish liquid
Activity	10 KU/mL or more

Properties

Stability	Stable at −20℃ for at least six months
Molecular weight	40 kDa
Isoelectric point	5.45

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

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

$2H_2O_2$ + 4-Aminoantipyrine + EHSPT $\xrightarrow{\text{Peroxidase}}$ 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 conditions described below.

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)
B. 4-AA solution	0.5% (500 mg of 4-aminoantipyrine/100 mL of H2O) (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 ca. 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 H2O)
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**

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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)

Reference

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

The effect of different conditions on Lactate Oxidase

A.

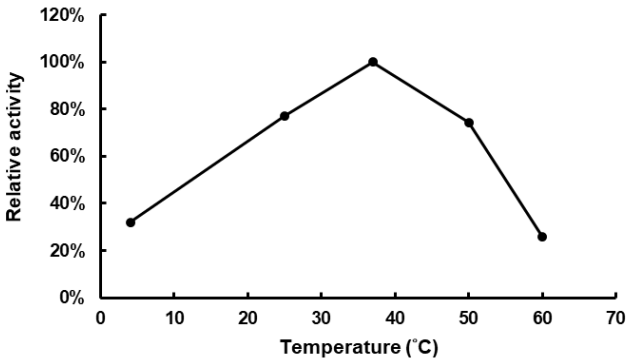

Figure A. Temperature activity of LOX. The enzyme reactions in 20 M K-Phosphate buffer, pH 7.5, were carried out under different temperatures.

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B.

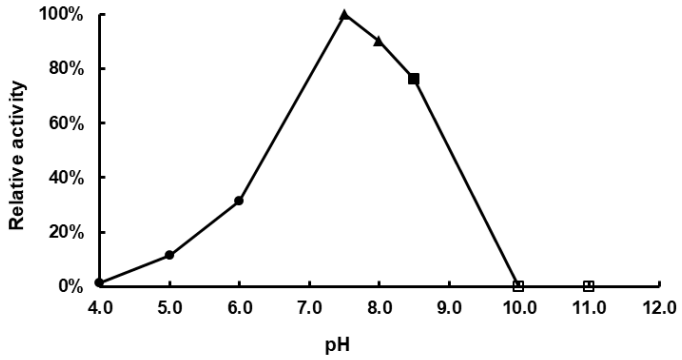

Figure B. pH activity of LOX. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.5-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

C.

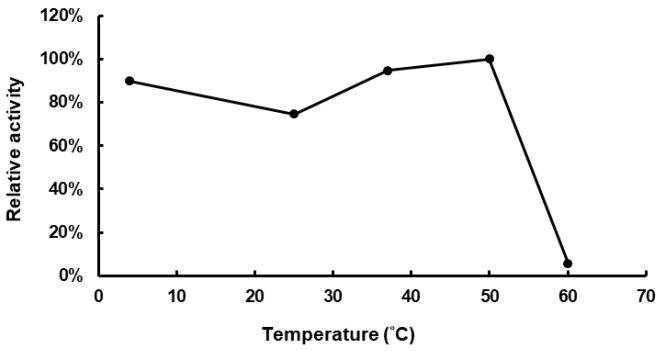

Figure C. Thermal stability of LOX. The enzyme was treated at different temperatures for 10 minutes. Final concentration: 10 U/mL

D.

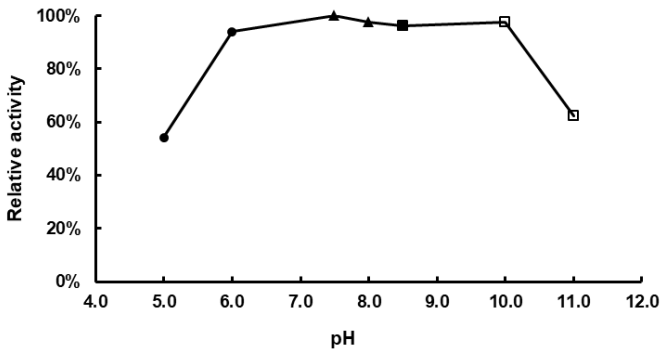

Figure D. pH stability of LOX. The enzyme was treated with different pH buffer condition at 25°C for 16 hours. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.5-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

Disclaimer

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