Lactate Oxidase

rp216190

Lactate Oxidase

(EC 1.1.3.2, Recombinant from microorganism)

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

Storage temperature

Stored at -20 ℃

Preparation and specification

Appearance	Yellowish amorphous powder,lyophilized
Protein purity	⩾90% (from SDS-PAGE)
Activity	⩾45.0 U/mg
Catalase	⩽0.001%
Creatinine amidohydrolase	⩽0.01%
Creatine amidohydrolase	⩽0.01%
ATPase	⩽0.001%

Properties

Source	Microorganisms	
Classification	EC1.1.3.2(Recombinant from microorganism)	
Molecular weight	42 kDa(SDS-PAGE)	
Isoelectric point	4.6	
Km Value	7.5 ×10^{-4} M(L-Lactate)	
Optimum pH	6.0-7.0	Fig. 1
Optimum temperature	50℃	Fig. 2
pH stability	pH 6.0-8.5(25 °C, 16 h)	Fig. 3
Thermal stability	Below 50℃(pH 7.5, 30 min)	Fig. 4
Storage stability	Store at -25~ -15 ℃ for 12 months could maintain more than 90% activity	Fig. 5

Applications

This enzyme is useful for enzymatic determination of lactic acid.

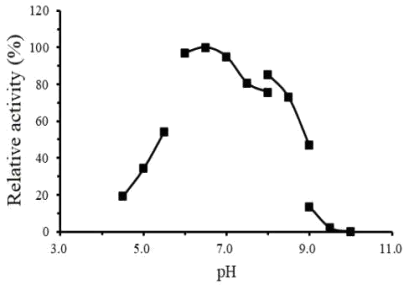


Fig. 1 Optimum pH

Buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

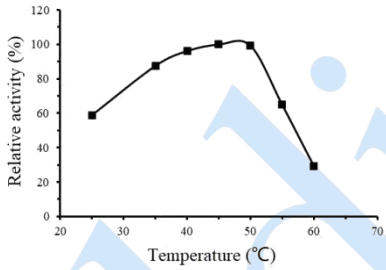


Fig. 2 Optimum temperature

Reaction in 20 mM K-phosphate buffer pH 7.0.
Enzyme concentration: 1 mg/mL

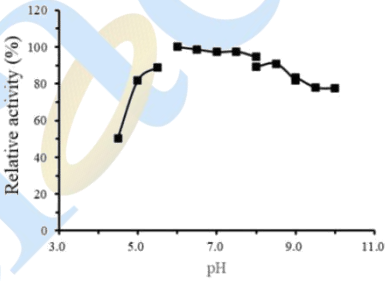


Fig. 3 pH Stability

25°C, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

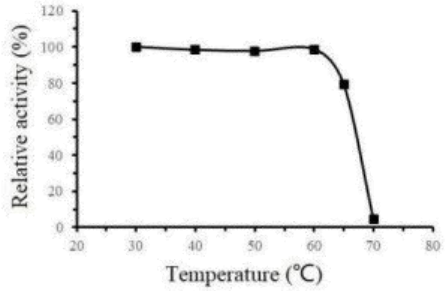


Fig. 4 Thermal stability

30 min-treatment with 50mM K-phosphate buffer pH 7.0.
Enzyme concentration: 1 mg/mL

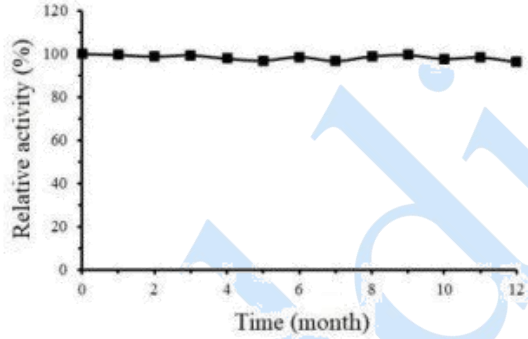
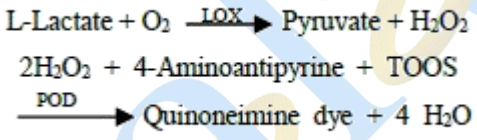


Fig.5 Storage stability (-25 ~ -15 ℃)

Assay principle



The amount of Quinoneimine dye produced by the reaction can be detected by spectrophotometer at 555 nm.

Unit definition

One unit (U) is defined as the amount of enzyme which produces 1 μmol of H_2O_2 per min under the conditions described below.

Reagents preparation

Reagent I: 0.2 M pH 6.5 potassium phosphate buffer.

Reagent II: 1kU/mL peroxidase (POD) solution. Reagent III: 50 mM 4-AA solution.

Reagent IV: 0.5 M DL- Lactic acid solution, pH 6.5.

Reagent V: 50 mM TOOS solution.

Enzyme diluent : 10 mM pH 7.0 potassium phosphate solution with 10 μM FAD. Samples: dilute the enzyme with enzyme diluent to 0.05 – 0.2 U/mL.

Prepare reaction mixture as follows:

Reagent I	10 ml
Reagent II	0.25 mL
Reagent III	1.5 mL
Reagent IV	5 mL
Reagent V	1.5 mL

Add ddH_2O to 50 ml

Procedure

1.Add 1 mL of the reaction mixture to 1 mL cuvette.

2.Heat the reaction mixture at 37 °C for 5 min.

3.Add 20 μL of the enzyme solution to the cuvette and mix.

4.Record the ΔAs at 555 nm in 1 minute in a spectrophotometer thermostated at 37 ℃.

* At the same time, measure the blank rate ΔAb by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution.ΔA = ΔAs - ΔAb

Calculation

$$Volume\ activity\ (U/mL) = \frac{\Delta A \times 1.02 \times df}{39.2 \times 0.02 \times 1 \times 1/2} = \Delta A \times 2.602 \times df$$

$$Weight\ activity\ (U/mg) = Volume\ activity \times 1/C$$

1.02: Total volume of reaction solution (mL) 0.02: Volume of enzyme solution (mL)

1: Light path length (cm)

1/2: 1mol H_2O_2 will react to 1/2 mol Quinoneimine dye

df: Dilution multiple

C: Enzyme concentration (mg/mL)

39.2: Millimolar extinction coefficient of quinoneimine dye under 555nm ($cm^2/\mu mol$)