Ketoamine oxidase (KAOD)

rp216165

Preparation and specification

Appearance	Yellow amorphous powder,lyophilized
Protein purity	⩾90% (from SDS-PAGE)
Activity	⩾4 U/mg solid
Catalase	⩽0.001%
ATPase	⩽0.005%
Glucose oxidase	⩽0.03%
Cholesterol oxidase	⩽0.003%

Properties

EC number	1.5.3(Recombinant from microorganism)	
Molecular weight	55 kDa (SDS-PAGE)	
Isoelectric point	6.0	
Michaelis constants	5.0 × 10^{-4} M（fructosyl-Ala）	
Inhibitors	Hg^{2+}, Pb^{2+}	
Optimum pH	7.7	Fig. 1
Optimum temperature	42℃	Fig. 2
pH stability	5.0-9.5 (25 ℃, 16 h)	Fig. 3
Thermal stability	Below 40 ℃ (pH 8.0, 30 min)	Fig. 4
Storage stability	At least one year at -20℃	Fig. 5
Stabilizers	Glycerol, Trehalose	

Applications

The enzyme is useful for the determination of fructosyl-L-amino acid.

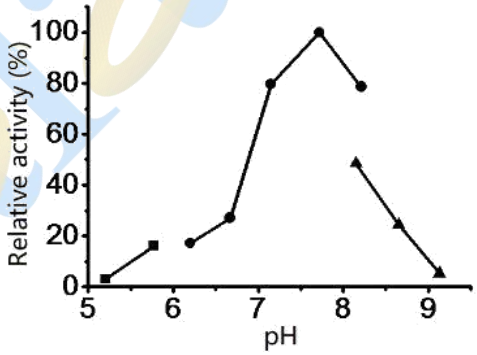


Fig. 1 Optimum pH

Buffer solution: pH 5.2-5.8, acetate buffer; pH 6.2-8.2,Na-phosphate; pH 8.2-9.2, Tris-HC1.

Enzyme concentration: l mg/ml

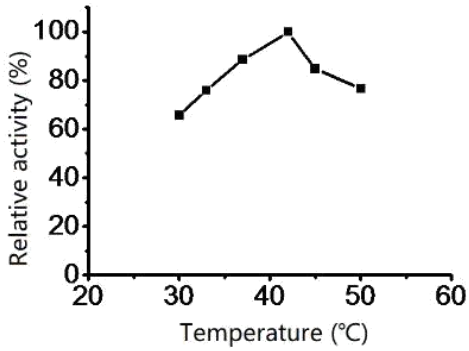


Fig. 2 Optimum temperature

Reaction in 20 mM Tris-HCl buffer, pH 8.0.Enzyme concentration: 1 mg/ml

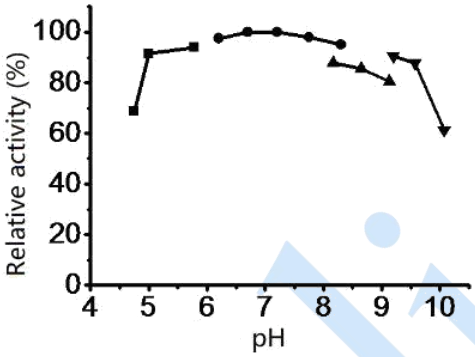


Fig. 3 pH Stability

25°C,16 h-treatment with 50mM buffer solution: pH4.5-6.0, acetate buffer; pH6.5-8.0, Na-phosphate; pH8.0-9.0,Tris-HIC1 pH9.0-10.0, Glycine-NaOH. Enzyme concentration:1mg/ml

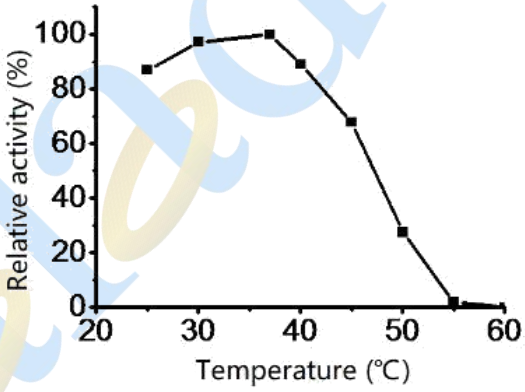


Fig. 4 Thermal stability

30 min-treatment with 100 mM phosphate buffer, pH 8.0 Enzyme concentration: 1 mg/mL

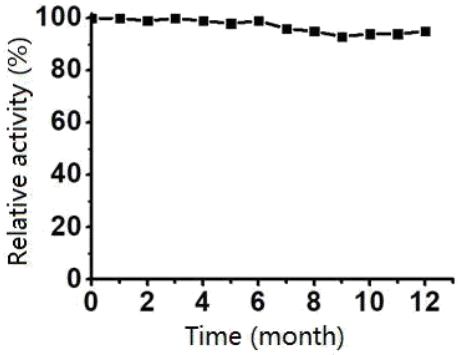
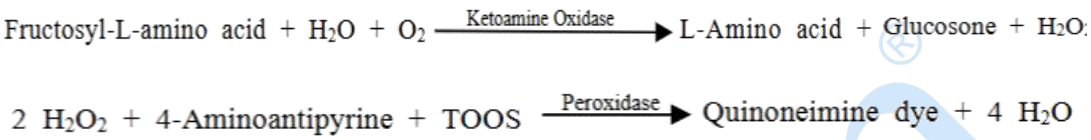


Fig.5 Storage stability (-20℃)

Assay principle



The assay is based on the increase in absorbance at 555 nm as the formation of quinoneimine dye proceeds in the forward reactions.

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: 0.1 M potassium phosphate, pH 8.0.
Reagent II: 1 kU/mL POD.
Reagent III: 50 mM TOOS solution.
Reagent IV: 50 mM 4-AA solution.
Reagent V: 200 mM fructosyl-valine solution.
Enzyme diluent: 20 mM Tris-HCl, pH 8.0.
Sample: Dilute the sample to 0.02-0.2 U/mL by Enzyme diluent.
Reaction mixture:

Reagent I	10 mL
Reagent II	0.1 mL
Reagent III	1 mL
Reagent IV	1 mL
Reagent V	10 mL
Pure water	Set to 100mL

Procedure

1.Add 0.98 mL reaction mixture to the cuvette.

2.Preincubate the reaction mixture at 37 ℃ for 5 min.

3.Add 0.02 mL the enzyme solution in the reaction mixture.

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

At the same time, measure the blank rate ΔA_b by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution. $\Delta A = \Delta A_s - \Delta A_b$

Calculation

$$\text{Volume activity (U/mL)} = \frac{\Delta A \times Vt \times df}{39.2 \times 1/2 \times t \times Vs \times 1.0} = \Delta A \times 2.551 \times df$$

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

Vt: Total volume (1.0 mL)

Vs: Enzyme volume (0.02 mL)

t: reaction time (1 min)

df: dilution factor

C: Enzyme concentration (mg/mL)

1.0: Light path length (cm)

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

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

References

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