Ketoamine Oxidase (TBP0221)

Catalog	Unit Size
TBP0211-500u	500u
TBP0211-2ku	2ku

Description

Ketoamine Oxidase is a highly specific oxidative enzyme that catalyzes the conversion of ketoamine substrates with excellent activity and selectivity. Its robust stability, broad substrate tolerance, and compatibility with mild reaction conditions make it a valuable tool for biochemical studies, pathway elucidation, and biocatalytic method development. The enzyme supports applications in metabolic research, analytical assays, and the synthesis of structurally defined intermediates.

Ketoamine oxidase (EC 1.5.3, from Microorganism)

Fructosyl-L-amino acid + H_2O + O_2 ⟶ L-Amino acid + Glucosone + H_2O_2

Specifications

Appearance	Yellow amorphous powder, lyophilized	
Protein purity	≥90%	
Activity	≥4 U/mg solid	
Catalase	≤0.001%	
ATPase	≤0.005%	
Glucose oxidase	≤0.03%	
Cholesterol oxidase	≤0.003%	
EC number	1.5.3 (Recombinant from microorganism)	
Molecular weight	55 kDa	
Isoelectric point	6	
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 ℃, 16h)	Fig. 3
Thermal stability	Below 40 ℃ (pH 8.0, 30min)	Fig. 4
Storage stability	At least one year at -25～-15 ℃	Fig. 5
Stabilizers	Glycerol, Trehalose	

Applications

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

Ketoamine Oxidase (TBP0221)

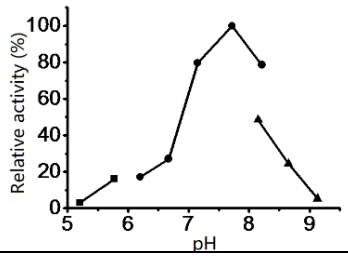

Fig. 1 Optimum pH

Buffer solution: pH 5.2-5.8, acetate buffer; pH 6.2-8.2, Na-phosphate; pH 8.2-9.2, Tris-HCl.
Enzyme concentration: 1 mg/mL

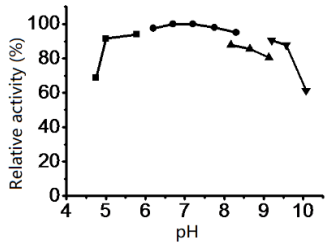

Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-6.0, acetate buffer; pH 6.5-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

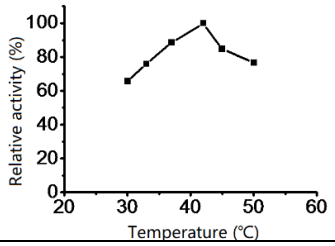

Fig. 2 Optimum temperature

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

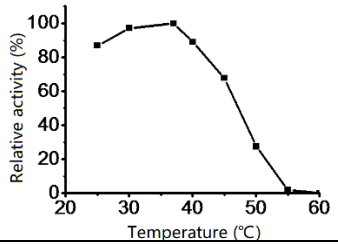

Fig. 4 Thermal stability

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

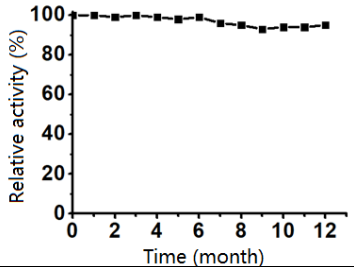

Fig.5 Storage stability (-25～-15 ℃)

Assay principle

Fructosyl-L-amino acid + H_2O + O_2 $\xrightarrow{\text{Ketoamine Oxidase}}$ L-Amino acid + Glucosone + H_2O_2

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

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-Ala 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. $\Delta A = \Delta As - \Delta A_b$

Calculation

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

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

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 ($cm^2/\mu mol$)

For research use only.