Fructosyl Peptide Oxidase (TBP0220)

Catalog	Unit Size
TBP0220-500u	500u
TBP0220-2ku	2ku

Description

Fructosyl Peptide Oxidase (FPOX) is a specialized oxidase used for the determination of glycated proteins and glycated peptides. It specifically recognizes and oxidizes fructosyl-peptide structures, generating detectable hydrogen peroxide during the reaction. This enzyme offers advantages such as high stability, strong selectivity, and convenient use. FPOX provides reliable analytical support for clinical diagnostics and research, making it an important enzymatic tool for studies of glycated proteins.

Fructosyl peptide oxidase (EC 1.5.3, from Microorganism)

Specifications

Appearance:	Yellow amorphous powder, lyophilized	
Protein purity:	≥90%	
Activity:	≥4 U/mg solid	
Catalase:	≤0.01%	
ATPase:	≤0.005%	
Glucose oxidase:	≤0.03%	
Cholesterol oxidase:	≤0.003%	
EC number:	1.5.3 (Recombinant from microorganism)	
Molecular weight:	60 kDa	
Isoelectric point:	6.4	
Michaelis constants:	4.0×10^{-3} M (fructosyl-Val-His)	
Inhibitors:	Hg^{2+}, Pb^{2+}	
Optimum pH:	pH 6.5-7.5	Fig. 1
Optimum temperature:	37 ℃	Fig. 2
pH stability:	pH 6.5-9.5 (25 ℃, 16 h)	Fig. 3
Thermal stability:	Below 40 ℃ (pH 8.0, 30 min)	Fig. 4
Storage stability:	At least one year at -25 ~ -15 ℃	Fig. 5
Stabilizers:	Glycerol, trehalose	

Data

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

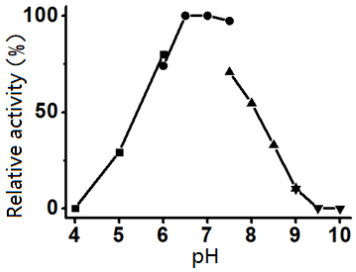

Fig. 1 Optimum pH

Buffer solution: pH 4.5-6.0, Acetate; pH 6.0-7.5, Na-phosphate; pH 7.5-9.0, Tris-HCl; pH 9.0-10, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

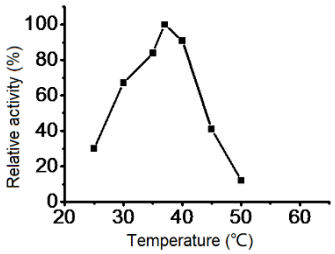

Fig. 2 Optimum temperature

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

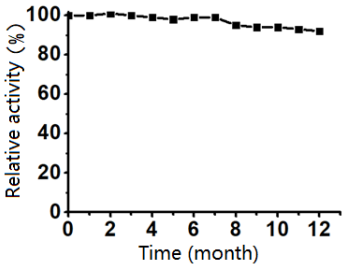

Fig.5 Storage stability (-25～-15 ℃)

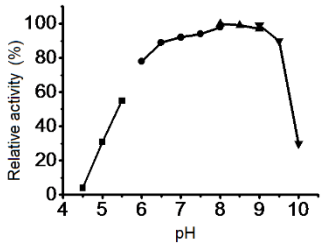

Fig. 3 pH Stability

25 ℃, 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

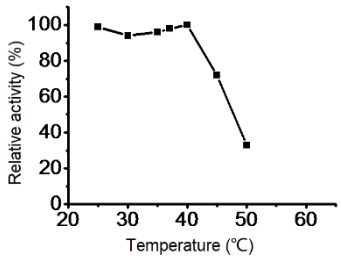

Fig. 4 Thermal stability

60 min-treatment with 20 mM Tris-HCl buffer, pH 8.0.
Enzyme concentration: 1 mg/mL

Assay principle

$$\text{Fructosyl-peptide} + H_2O + O_2 \xrightarrow{\text{Fructosyl-peptide oxidase}} \text{Peptide} + \text{Glucosone} + H_2O_2$$

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

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-valine solution.
Enzyme diluent: 20 mM Tris-HCl，pH 8.0.
Sample: Dilute the sample to 0.2-0.5 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

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

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

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/μmol)

For research use only.