

Fructosyl-amino Acid Oxidase (FAOD-E)

from recombinant *E. coli*

Fructosyl-L-amino acid : oxygen oxidoreductase, EC 1.5.3.



SPECIFICATION

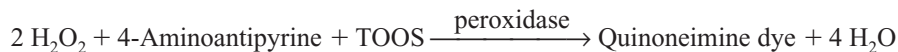
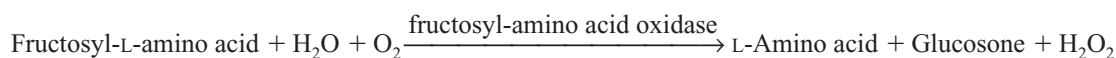
Appearance	yellow lyophilizate
Activity	≥ 4 U/mg lyophilizate
Stabilizer	Trehalose
Storage	at -20°C

PROPERTIES

Molecular weight	ca. 45 kDa (gel filtration)
Structure	monomer of 50 kDa (SDS-PAGE)
Michaelis constant	2.2×10^{-4} M (<i>N</i> ^ε -fructosyl-L-lysine)
pH Optimum	8.0–8.5 (Fig. 1)
pH Stability	6.0–8.5 (Fig. 2)
Optimum temperature	35–40°C (Fig. 3)
Thermal stability	below 30°C (Fig. 4)
Stability (liquid form)	stable at 25°C for at least two weeks (Fig. 5)
Stability (powder form)	stable at 37°C for at least three weeks (Fig. 6)
Inhibitors	Ag ⁺ , Cu ²⁺
Specificity	<i>N</i> ^ε -fructosyl-L-lysine (100), fructosyl-L-valine (65) fructosyl-glycine (30)

ASSAY PROCEDURE

Principle



The appearance of quinoneimine dye is measured spectrophotometrically at 555 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which produces 1 μmol of hydrogen peroxide per min at 30°C and pH 8.0 under the conditions described below.

Reagents

- Potassium phosphate buffer, 0.1 M; pH 8.0: mix 0.1 M KH_2PO_4 solution and 0.1 M K_2HPO_4 solution to make a pH 8.0 solution.
- TOOS solution, 0.5%: 125 mg of TOOS (*N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-*m*-toluidine)/25 ml of distilled water.
- Peroxidase (POD)-4-aminoantipyrine (4-AA) solution: dissolve 5 mg of POD (200 guaiacol U/mg) in 800 ml of potassium phosphate buffer (Reagent A), then add 100 mg of 4-AA and dilute with potassium phosphate buffer (Reagent A) to 1000 ml.
- Fructosyl-L-valine solution, 150 mM: 834 mg of fructosyl-L-valine/20 ml of distilled water.
- Enzyme dilution buffer: 10 mM potassium phosphate buffer, pH 8.0, containing 0.06% *n*-octhyl- β -D-thioglucoside.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.05–0.18 U/ml in ice-cold enzyme dilution buffer (Reagent E) immediately before measurement.

Procedure

- Pipette the following reagents into a cuvette (light path: 1 cm).

0.1 ml	TOOS solution	(Reagent B)
2.7 ml	POD-4-AA solution	(Reagent C)
0.1 ml	sample	
- Equilibrate at 30°C for about 5 min.
- Add 0.1 ml of fructosyl-L-valine solution (Reagent D) and mix.
- Record the increase of absorbance at 555 nm in a spectrophotometer thermostated at 30°C, and calculate the ΔA per min using the linear portion of the curve (ΔA_5).
The blank solution is prepared by adding distilled water instead of fructosyl-L-valine solution (Reagent D) (ΔA_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_s - \Delta A_0) \times 3.0(\text{ml}) \times df}{39.2 \times 1/2 \times 0.1(\text{ml})} = \Delta A \times 1.53 \times df$$

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

39.2 : Millimolar extinction coefficient of quinoneimine dye under the assay conditions ($\text{cm}^2/\mu\text{mol}$)

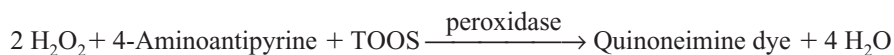
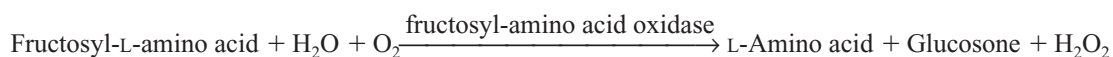
1/2 : Factor based on the fact that 1 mol of hydrogen peroxide produces 1/2 mol of quinoneimine dye

df : Dilution factor

C : Content of fructosyl-amino acid oxidase preparation in sample (mg/ml)

APPLICATIONS

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

**REFERENCES**

- Horiuchi, T. *et al.*, *Agric. Biol. Chem.*, **53**, 103–110 (1989).
 Sakaue, R. *et al.*, *Biosci. Biotechnol. Biochem.*, **66**, 1256–1261 (2002).
 Hirokawa, K. and Kajiyama, N., *Biosci. Biotechnol. Biochem.*, **66**, 2323–2329 (2002).
 Sakaue, R. and Kajiyama, N., *Appl. Environ. Microbiol.*, **69**, 139–145 (2003).

EXPERIMENTAL DATA

Fig. 1 pH Optimum

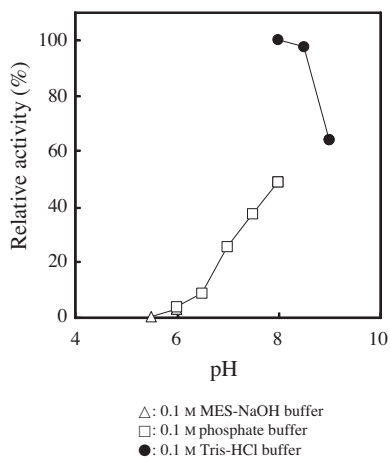


Fig. 2 pH Stability

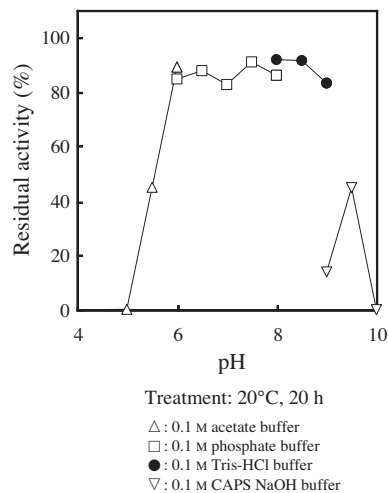
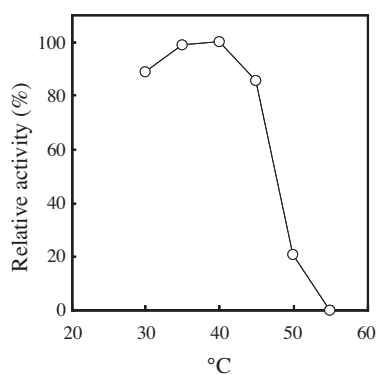
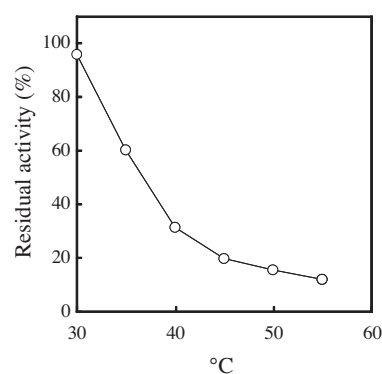


Fig. 3 Optimum temperature



Buffer: 0.1 M phosphate buffer, pH 8.0

Fig. 4 Thermal stability



Treatment: 0.1 M phosphate buffer, pH 8.0, 15 min

Fig. 5 Stability (liquid form) at 5°C and 24°C

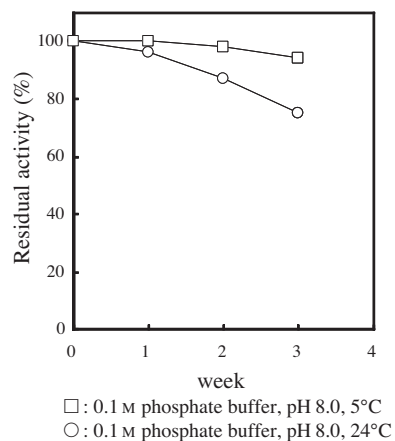


Fig. 6 Stability (powder form) at 37°C

