FRUCTOSYLAMINE OXIDASE [FOD Ⅲ]

from Microorganism
(Ketoamine oxidase, EC 1.5.3)

D-Glucose + acceptor ⟶ D-glucono-1,5-lactone + reduced acceptor

F

Preparation and Specification

Appearance : Yellowish lyophilized powder
Specific activity : More than 10 U/mg solid

Properties

Molecular weight : 49 kDa (SDS-PAGE)
Michaelis constants : 1.61 × 10^{-3}M (Fructosyl valylhistidine)
Optimum pH : See Figure 1
pH stability : See Figure 2
Optimum Temperature : See Figure 3
Thermal Stability : See Figure 4
Substrate specificity : See Table 1
Effect of various chemicals on FOD Ⅲ activity : See Table 2 and Table 3
Effect of various chemicals on FOD Ⅲ stability : See Table 4

Applications for Diagnostic Test

This enzyme is useful for the measurement of the glycated hemoglobin (HbA1c) in human whole blood.

Fig.1 Optimum pH

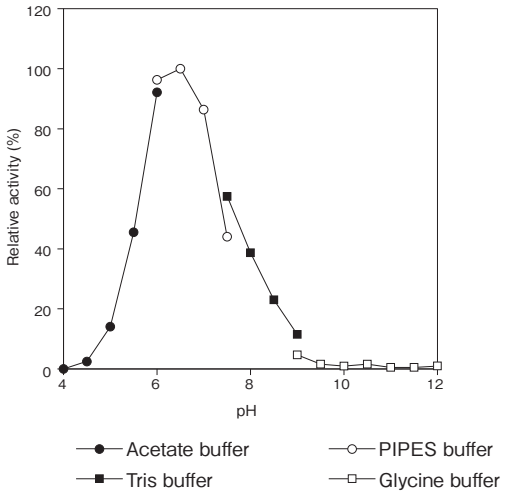


Fig.2 pH Stability

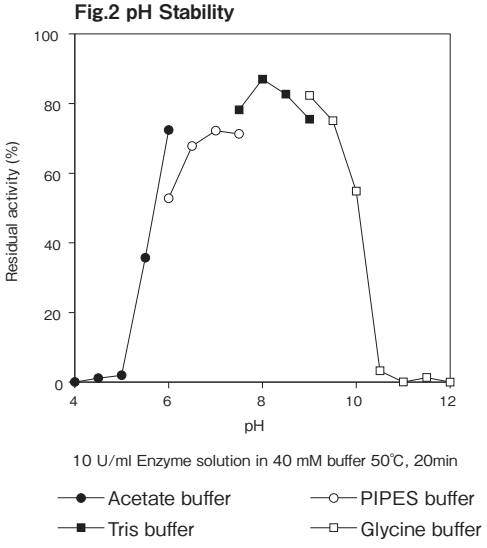
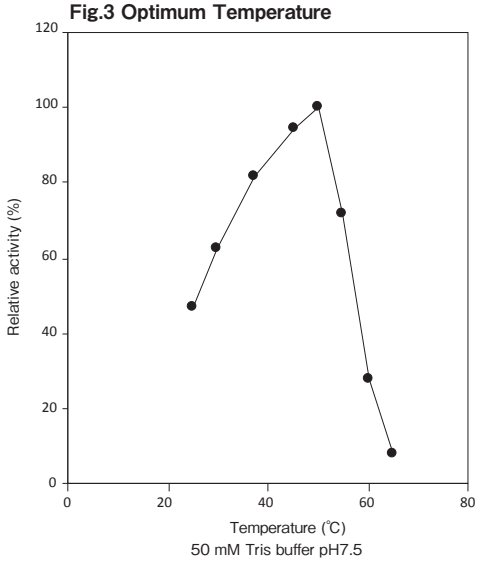
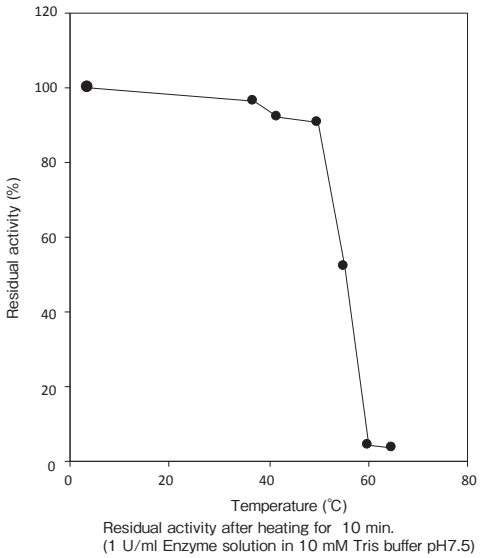


Fig.3 Optimum Temperature



50 mM Tris buffer pH7.5

Fig.4 Thermal Stability



Residual activity after heating for 10 min.
(1 U/ml Enzyme solution in 10 mM Tris buffer pH7.5)

F

Table 1. Substrate specificity

Substrate	Relative activity (%)
Fructosyl Valine	436.0
Fructosyl Valine-Histidine	100.0
Fructosyl Valine-Leucine	0.9
Fructosyl Valine-Histidine-Leucine	0.0
Fructosyl Valine-Histidine-Leucine-Threonine	0.0
Fructosyl Valine-Histidine-Leucine-Threonine-Proline	0.0
Fructosyl Valine-Leucine-Threonine-Proline-Leucine	0.0

Table 2. Effect of various chemicals on FODⅢ activity

Additive	Concentration	Relative activity (%)
None	-	100
MgCl2	0.5mM	101
MnCl2	0.5mM	103
CaCl2	0.5mM	103
LiCl	0.5mM	103
NaCl	0.5mM	110
CoCl2	0.5mM	12
FeCl2	0.5mM	44
KCl	0.5mM	107
EDTA	1.0mM	109
TritonX-100	0.1%	100
Sodium cholate	0.1%	98
Tween 80	0.1%	103
Tween 60	0.1%	103
Briji 35	0.1%	105

Table 3. Effect of various chemicals on FODⅢ activity

Additive	Concentration	Relative activity (%)
None	-	100
KCl	1mM	101
	20mM	95
	100mM	84
NaCl	1mM	97
	20mM	93
	100mM	86
	250mM	68
Sodium lauryl sulfate	0.01%	94
	0.03%	77
	0.05%	3
	0.10%	0
Ethylene glycol	1%	89
	2%	76
	5%	57
	10%	39
	20%	17
Dimethyl Sulfoxide	1%	87
	2%	77
	5%	61
	10%	42
	20%	22
2-Hydroxypropyl-β-cyclodextrin	1%	97
	3%	92
	5%	111

Table 4. Effect of various chemicals on FODⅢ stability

Additive	Residual activity (%)
None (40mM Tris-HCl pH7.5)	71
+ 5mM EDTA	85
+ 250mM KCl	85
+ 250mM NaCl	89
+ 20mM Sodium glutamate	85
+ 20% Sucrose	94
+ 20% Ethylene glycol	86
+ 20% Glycerol	91
+ 0.1% Triton X-100	64
+ 4% Sorbitol	99
+ 0.1% Briji 35	65
+ 0.1% Tween 60	74
+ 0.002mM Flavin adenine dinucleotide	76
+ 0.02mM Flavin adenine dinucleotide	76
+ 0.002mM Flavin mononucleotide	75
+ 0.02mM Flavin mononucleotide	74
+ 10mM NH4Cl	77

Residual activity after heating for 50 ℃, 10 min.
(3U/ml Enzyme solution)

F

Assay

■ Principle

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

1-deoxyfructosyl-valinyl-histidine + O_2 + H_2O
$\xrightarrow[\text{FOD Ⅲ}]{}$ Glucosone + Valinyl-histidine + H_2O_2

$2H_2O_2$ + 4-AA + TOOS $\xrightarrow[\text{POD}]{}$ quinoneimine dye + $4H_2O$

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μ mole of deoxyfructosyl-valinyl-histidine to H_2O_2 per minute at 37 ℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture
   50mM Tris-HCl buffer pH 7.5 containing 1.0mM 1-deoxyfructosyl-valinyl-histidine and 0.03% 4-AA and 0.02% TOOS and 5.0U/mL POD
2. Reaction stopper
   0.5% SDS solution
3. Enzyme dilution buffer
   10mM Tris-HCl buffer pH7.5
4. Reagents
   Tris (hydroxymethyl) aminomethane: Sigma #T-1503
   1-deoxyfructosyl-valinyl-histidine: Peptide Institute. Inc.
   4-AA (4-Aminoantipyrine) : nacalai tesque #01907-52
   TOOS (N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate) : DOJINDO LABORATORIES #OC13
   SDS (Sodium lauryl sulfate) : nacalai tesque #31606
   POD (Peroxidase) : Sigma Type Ⅱ #P-8250

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 0.5 ml of reaction mixture into a small test tube and preincubate at 37 ℃.
2. After 5 min, add 10 μl of enzyme solution and mix to start the reaction at 37 ℃.
3. At 5min after starting the reaction, add 1.0 ml of reaction stopper and mix to stop the reaction.
   ※ IIn the case of a test blank, add 10 μl of enzyme dilution buffer in place of enzyme solution after stopping the reaction.
4. Measure the absorbance at 555 nm.
   Absorbance sample : As
   blank : Ab
   △ A = (As − Ab) ≦ 0.050 ～ 0.800 Abs

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/5\text{min}}{39.2 \times 1/2} \times \frac{1.51}{0.01} \times \frac{1}{X}$$

$$= \triangle A/\text{min} \times 1.541 \div X$$

39.2 : millimolar extinction coefficient of quinoneimine dye at 555 nm (cm^2/ μmole)
1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye
1.51 : final volume (ml)
0.01 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at -20 ℃ in the presence of a desiccant is recommended.

FOD Ⅲ活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液
   1.0mM 1-deoxyfructosyl-valinyl-histidine、0.03% 4-AA、0.02% TOOS、5.0U/ml POD を含む 50mM トリス -HCl 緩衝液 pH7.5
2. 反応停止液
   0.5% SDS 溶液
3. 酵素溶解希釈溶液
   10mM トリス -HCl 緩衝液 pH7.5
4. 試薬
   トリス (ヒドロキシメチル) アミノメタン:シグマ製 #T-1503
   1-deoxyfructosyl-valinyl-histidine:ペプチド研究所製
   4-AA (4- アミノアンチピリン) :ナカライテスク製 特級 #01907-52
   TOOS:同仁化学製 #OC13
   SDS (ドデシル硫酸ナトリウム):ナカライテスク製 #31606
   POD (パーオキシダーゼ):シグマ製 Type Ⅱ #P-8250

Ⅱ. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ．測定操作法

1. 小試験管に反応試薬混合液 0.5ml ずつを正確に分注し、37℃で予備加温する。
2. 5 分経過後、酵素試料液 10 μl を正確に加えて混和し、37℃で反応を開始する。
3. 5 分経過後、反応停止液 1.0ml を加えて混和し、反応を停止する。
   ※盲検は反応停止後に酵素試料液 10 μl を加える。
4. 555nm における吸光度を測定する。
   求められた吸光度を試料液については As、盲検液については Ab とする。
   ※吸光度範囲　ΔA =（As − Ab）= 0.050〜0.800Abs

Ⅳ．計算

$$\text{活性 (U/mg)} = \frac{\Delta A/5\text{min}}{39.2 \times 1/2} \times \frac{1.51}{0.01} \times \frac{1}{X}$$

$$= \Delta A/\text{min} \times 1.541 \div X$$

39.2 ：キノンイミン色素の 555nm におけるミリモル分子吸光係数（$cm^2/\mu mol$）
1/2 ：H_2O_2 2 モルからキノンイミン色素 1 モルが生成することによる係数
1.51 ：反応総液量（ml）
1.51 ：反応に供した酵素試料液量（ml）
X　：酵素試料液中の検品濃度（mg/ml）