(Diagnostic Reagent Grade)

T-191

FRUCTOSYLAMINE OXIDASE [FOD Ⅲ]

from Microorganism
(Ketoamine oxidase, EC 1.5.3)

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

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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

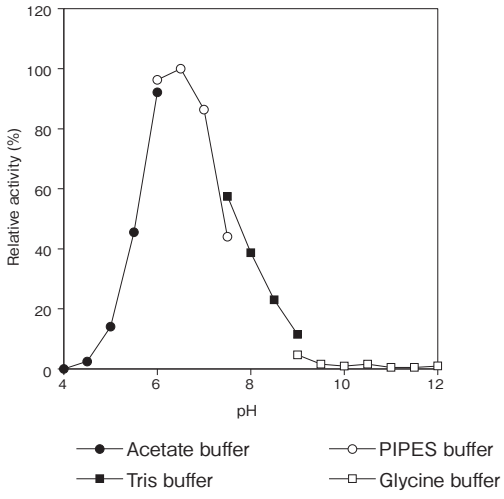

Fig.2 pH Stability

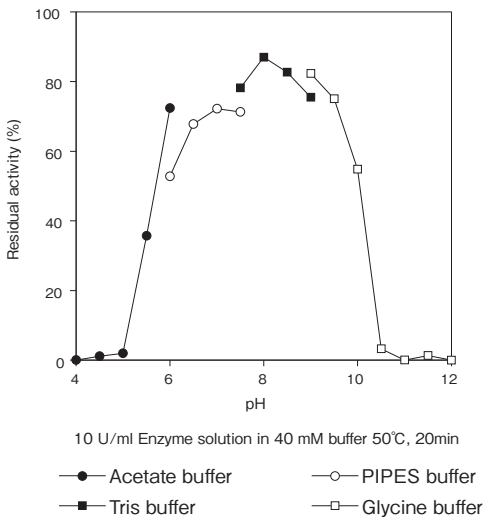

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Fig.3 Optimum Temperature

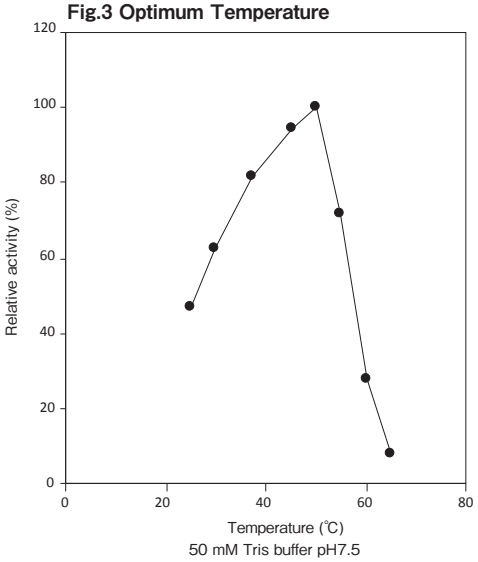

Fig.4 Thermal Stability

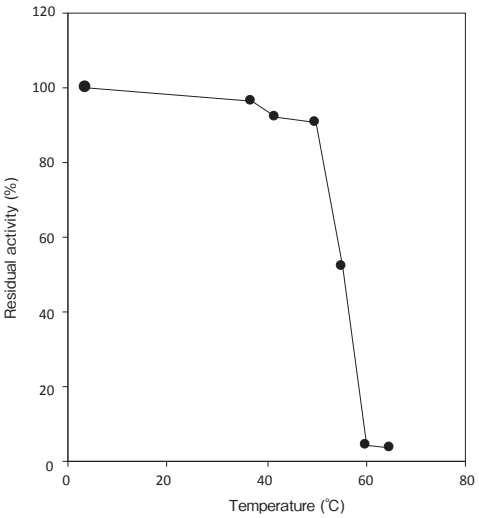

Table 1. Substrate specificity

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)

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

$$\xrightarrow[\text{FOD Ⅲ}]{} \text{Glucosone + Valinyl-histidine + } H_2O_2$$

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

■ 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）

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