●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

XANTHINE OXIDASE

from Microorganism

Xanthine:oxygen oxidoreductase (EC 1.1.3.22)

$$\text{Hypoxanthine} + O_2 + H_2O \longrightarrow \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{Uric acid} + H_2O_2$$

PREPARATION and SPECIFICATION

Appearance	: Reddish brown amorphous powder, lyophilized	
Activity	: Grade Ⅱ 10U/mg-solid or more	
Contaminants	: Catalase	≤5%
	Adenosine deaminase	$\leq 1.0\times10^{-3}$%
	Uricase	$\leq 1.0\times10^{-3}$%
	Phosphatase	$\leq 1.0\times10^{-3}$%
	Purine-nucleoside phosphorylase	$\leq 5.0\times10^{-3}$%
Stabilizers	: Sodium glutamate, BSA	

PROPERTIES

Stability	: Stable at −20℃ for at least year	(Fig.1,2)
Molecular weight	: approx. 160,000	
Isoelectric point	: 4.0±0.1	
Michaelis constants	: 4.5×10^{-5}M (Xanthine)	
	7.6×10^{-5}M (Hypoxanthine)	
Inhibitors	: Reducing agents, Hg^{++}, Ag^{+},MIA	
Optimum pH	: 7.5−8.0	(Fig.3)
Optimum temperature	: 65℃	(Fig.4)
pH Stability	: pH 6.5−9.0 (25℃, 15hr)	(Fig.5)
Thermal stability	: below 55℃ (pH 8.0, 30min)	(Fig.6)
Substrate specificity	: (Table 1)	
Effect of various chemicals	: (Table 2)	

APPLICATIONS

This enzyme is useful for enzymatic determination of inorganic phosphorus, 5'-nucleotidase and adenosine deaminase when coupled with Purine-nucleoside phosphorylase (PNP-301) and uricase (UAO-201, UAO-211).

ASSAY

Principle:

Xanthine + O_2 + H_2O $\xrightarrow{\text{xanthine oxidase}}$ Uric acid + H_2O_2

The appearance of uric acid is measured at 293nm by spectrophotometry.

Unit definition:

One unit causes the formation of one micromole of uric acid per minute under the conditions described below.

Method:

Reagents

A. Tris-HCl buffer, pH 7.5 : 0.1M
B. Sodium hydroxide solution : 0.025M
C. Xanthine solution : 10mM [Dissolve 15.2mg of xanthine (MW=152.11) in 10ml solution (B)]
D. Oxonic acid potassium salt solution : 1mM (Dissolve 9.75mg of oxonic acid・K salt in 50ml H_2O)
E. Enzyme diluent : 50mM Tris-HCl buffer, pH 7.5

Procedure

1. Prepare the following reaction mixture in a cuvette (d=1.0cm) and equilibrate at 37℃ for about 5 minutes.

2.24ml	Tris-HCl buffer, pH 7.5	(A)
0.08ml	Xanthine solution	(C)
0.08ml	Oxonic acid solution	(D)

Concentration in assay mixture	
Tris-HCl buffer	ca.89.6 mM
Xanthine	0.32mM
Oxonic acid	32 μM

2. Add 0.1ml of the enzyme solution* and mix by gentle inversion.
3. Record the increase in optical density at 293nm against water for 3 to 4 minutes in a spectrophotometer thermostated at 37℃, and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test).

   At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent (E) is added instead of the enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent (E), dilute to 0.1−0.2U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta\text{OD/min}\ (\Delta\text{OD test} - \Delta\text{OD blank}) \times \text{Vt} \times \text{df}}{12.5 \times 1.0 \times \text{Vs}}$$

$$= \Delta\text{OD/min} \times 2.0 \times \text{df}$$

Weight activity (U/mg) = (U/ml) × 1/C

Vt : Total volume (2.5ml)
Vs : Sample volume (0.1ml)
12.5 : Millimolar extinction coefficient of uric acid under the assay condition (㎠/micromole)
1.0 : Light path length (cm)
df : Dilution factor
C : Enzyme concentration in dissolution (c mg/ml)

Table 1. Substrate Specificity of Xanthine oxidase (color-system)

Substrate(100mM)	Relative activity
Xanthine	100
Hypoxanthine	18.7
Purine	6.4
Guanine	18.3
Adenosine	0

Table 2. Effect of Various Chemicals on Xanthine oxidase

[The enzyme dissolved in 50mM Tris-HCl buffer, pH 7.5 (2U/ml) was incubated with each chemical at 25℃ for 2hr.]

Chemical	Concn.(mM)	Residual activity
None	—	100%
Metal salt	2.0	
$MgCl_2$		78
$CaCl_2$		96
$Ba(OAc)_2$		102
$FeCl_3$		85
$CoCl_2$		100
$MnCl_2$		102
$ZnSO_4$		62
$CdCl_2$		34
$NiCl_2$		91
$CuSO_4$		18
$PbCl_2$		9.7
AgCl		0
$HgCl_2$		1.2

Chemical	Concn.(mM)	Residual activity
PCMB	2.0	24
MIA	2.0	1.1
NaF	2.0	14
NaN_3	20	25
EDTA	5.0	95
o-Phenanthroline	2.0	89
α,α'- Dipyridyl	1.0	81
Borate	20	89
IAA	2.0	24
NEM	2.0	91
Triton X-100	0.1%	99
Brij 35	0.1%	50
Tween 20	0.1%	106
Span 20	0.1%	110
Na-cholate	0.1%	115
SDS	0.05%	111
DAC	0.05%	78

Ac, CH_3CO; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; EDTA, Ethylenediaminetetraacetate; IAA, Iodoacetamide; NEM, N-Ethylmaleimide; SDS, Sodium dodecyl sulfate; DAC, Dimethyl-benzyl-alkyl-ammonium chloride.

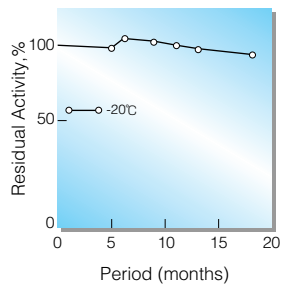

Fig.1. Stability (Powder form)

〔kept under dry conditions〕

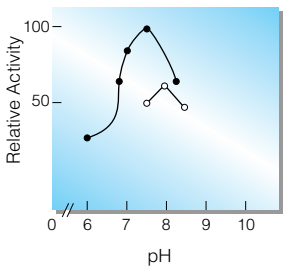

Fig.3. pH-Activity

〔37℃ in 4min-reaction in 50mM buffer solution
●:pH6.0-8.2 K-phosphate
○:pH7.5-8.5 Tris-HCl〕

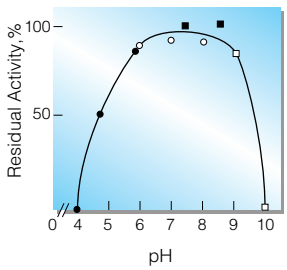

Fig.5. pH-Stability

〔25℃ 15hr with 50mM buffer solution
●:pH4.0-6.0 acetate
○:pH6.0-8.0 K-phosphate
■:pH8.0-9.0 Tris-HCl
□:pH9.0-10.0 Na-K carbonate〕

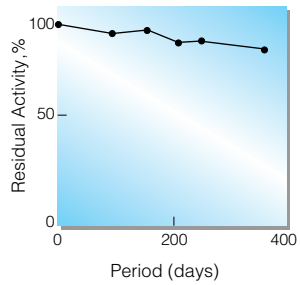

Fig.2. Stability (Liquid form)

〔in 50mM buffer solution 4℃,pH6.8
enzyme concn.:60U/ml〕

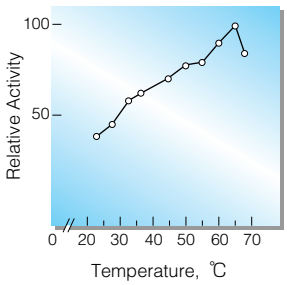

Fig.4. Thermal activity

〔in 50mM Tris-HCl buffer, pH8.0〕

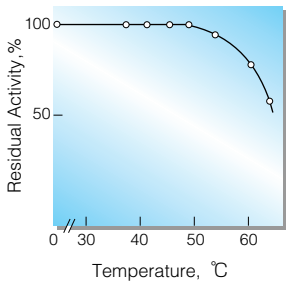

Fig.6. Thermal stability

〔30min-treatment with 50mM Tris-HCl buffer,pH8.0〕

活性測定法（Japanese）

1.原理

Xanthine＋O_2＋H_2O $\xrightarrow{\text{xanthine oxidase}}$ Uric acid＋H_2O_2

尿酸の生成量を293nmにおける吸光度の変化で測定する。

2.定義

下記条件で1分間に1マイクロモルの尿酸を生成する酵素量を1単位(U)とする。

3.試薬

A. 0.1M Tris-HCl緩衝液, pH7.5
B. 25mM NaOH水溶液
C. 10mMキサンチン水溶液〔15.2㎎のキサンチン(MW＝152.11)を10㎖のNaOHの水溶液(B)で加温溶解する。〕(4℃保存で2週間は使用可能)
D. 1mMオキソン酸・K水溶液〔9.75㎎のオキソン酸・K塩(MW＝195.18)を50㎖の蒸留水に溶解する。〕(4℃保存で2週間は使用可能)

酵素溶液：酵素標品を予め氷冷した50mM Tris-HCl緩衝液,pH7.5で溶解し,同緩衝液で0.1－0.2U/㎖に希釈して氷冷保存する。

4.手順

①下記反応混液をキュベット(d＝1.0cm)に調製し,37℃で約5分間予備加温する。

2.24㎖	Tris-HCl緩衝液	(A)
0.08㎖	キサンチン水溶液	(C)
0.08㎖	オキソン酸・K水溶液	(D)

②酵素溶液0.1㎖を添加し,ゆるやかに混和後,水を対照に37℃に制御された分光光度計で293nmの吸光度変化を求める(ΔODtest)。

③盲検は反応混液①に酵素溶液の代わりに酵素希釈液(50mM Tris-HCl緩衝液, pH7.5)を0.1㎖加え,上記同様に操作を行って,1分間当たりの吸光度変化を求める(ΔODblank)。

5.計算式

$$U/㎖ = \frac{\Delta OD/min\ (OD\ test - OD\ blank) \times 2.5(㎖) \times 希釈倍率}{12.5 \times 1.0 \times 0.1(㎖)}$$

$$= \Delta OD/min \times 2.0 \times 希釈倍率$$

U/㎎ ＝ U/㎖×1 / C

12.5 ： 尿酸のミリモル分子吸光係数(㎠/micromole)

1.0 ： 光路長(cm)

C ： 溶解時の酵素濃度(c ㎎/㎖)