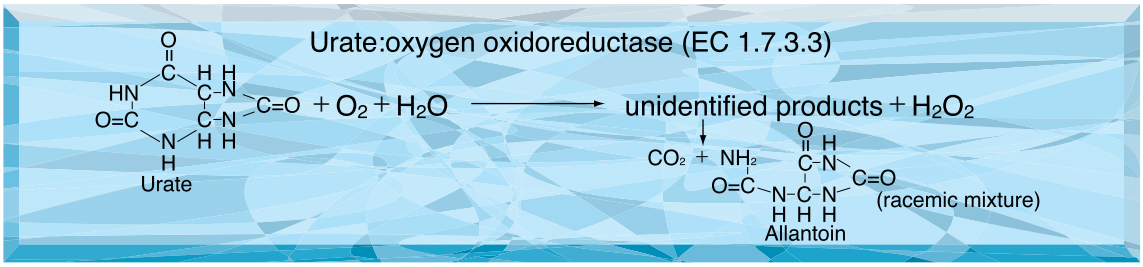
●TOYOBO ENZYMES●

(Diagnostic Reagent Grade)

URICASE

from Candida sp.



PREPARATION and SPECIFICATION

Appearance	: White amorphous powder, lyophilized
Activity	: Grade Ⅱ 4.0U/mg-solid or more (containing approx.20% of stabilizers)
Contaminant	: Catalase ≤1.0%
Stabilizers	: Borate, EDTA, nonionic detergents

PROPERTIES

Stability	: Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	: approx. 120,000	
Structure	: 4 subunits per molecule (Reactive SH groups are present in the enzyme molecule)	
Isoelectric point	: 5.4	
Michaelis constant	: 2.5×10^{-5}M (Uric acid)	
Inhibitors	: Heavy metal ions, cyanide,various urate analogs	
Optimum pH	: 8.5	(Fig.3)
Optimum temperature	: 40℃	(Fig.4)
pH Stability	: pH 7.0−11.0 (25℃, 20hr)	(Fig.5)
Thermal stability	: below 50℃ (pH 8.5, 10min)	(Fig.6)
Effect of various chemicals	: (Table 1)	

APPLICATIONS

This enzyme is useful for enzymatic determination of uric acid in clinical analysis.

ASSAY

Principle:

Uric acid + O_2 + $2H_2O$ $\xrightarrow{\text{uricase}}$ Allantoin + H_2O_2 + CO_2

The disappearance of uric acid is measured at 290nm by spectrophotometry.

Unit definition:

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

Method:

Reagents

A. Uric acid solution : 0.001% [Dilute the stock solution (0.01%) to 10-fold volume with 50mM borate buffer containing 0.001% Triton X-100 and 1.0mM EDTA, pH 8.5] (Should be prepared fresh) stock solution : 10mg uric and/100ml of above buffer (store at 0−5℃)

B. KOH solution : 20%

C. Enzyme diluent : 50mM borate buffer containing 0.001% Triton X-100 and 1.0mM EDTA, pH 8.5

Procedure

1. Prepare the following reaction mixture in a test tube and equilibrate at 25℃ for about 5 minutes.

   2.0ml Uric acid solution (A)

   0.5ml Distilled water

Concentration in assay mixture		
Borate buffer	42	mM
Uric acid	40	μM
EDTA	0.83	mM
Triton X-100	0.00083%	

2. Add 0.5ml of the enzyme solution* and mix by gentle inversion.
3. After exactly 5 minutes at 25℃, add 0.2ml of 20% KOH solution (B) to stop the reaction and measure the optical density at 290nm against water (OD test).

   At the same time, prepare the blank by first mixing the reaction mixture with 0.2ml of KOH solution after 5min-incubation at 25℃, followed by the addition of the enzyme solution (OD blank).

* Dissolve the enzyme preparation in ice-cold enzyme diluent (C) and dilute to 0.01−0.02U/ml with the same buffer and store on ice.

Calculation

Activity can be calculated by using the following formula :

$$\text{Volume activity (U/ml)} = \frac{\Delta OD\ (OD\ blank - OD\ test) \times Vt \times df}{12.2 \times 1.0 \times t \times Vs} = \Delta OD \times 0.105 \times df$$

Weight activity (U/mg) = (U/ml) × 1/C

Vt : Total volume (3.2ml)

Vs : Sample volume (0.5ml)

12.2 : Millimolar extinction coefficient of uric acid (㎠/micromole)

t : Reaction time (5 minutes)

1.0 : Light path length (cm)

df : Dilution factor

C : Enzyme concentration in dissolution (c mg/ml)

REFERENCES

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3) N.Kageyama et al.; *Eisei Kensa(Japanese), 19,* 338 (1969)
4) N.Kageyama et al.; *Eisei Kensa(Japanese), 18,* 59 (1969)
5) N.Kageyama; *Rirsho Kensa(Japanese), 16,* 891 (1972)
6) N.Kageyama; *Clin.Chim.Acta, 31,* 421 (1971)
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Table 1 Effect of Various Chemicals on Uricase

[The enzyme dissolved in 50mM borate buffer, pH 8.5 containing 0.001% Triton X-100 and 1.0mM EDTA (10U/ml) was incubated with each chemical at 25℃ for 1hr.]

Chemical	Concn.(mM)	Residual activity
None	—	100%
Metal salt	2.0	
$MgCl_2$		95
$CaCl_2$		91
$Ba(OAc)_2$		95
$FeCl_3$		88
$CoCl_2$		88
$MnCl_2$		84
$ZnCl_2$		69
$CdCl_2$		64
$NiCl_2$		79
$CuSO_4$		9.8
$Pb(OAc)_2$		74
$AgNO_3$		0
$HgCl_2$		0
2-Mercaptoethanol	2.0	101
PCMB	1.0	34
MIA	2.0	70
NEM	2.0	41
IAA	2.0	94
Hydroxylamine	2.0	95
EDTA	5.0	90
o-Phenanthroline	2.0	98
α,α'-Dipyridyl	1.0	99
Borate	50	90
Naf	2.0	87
NaN_3	2.0	96
Triton X-100	0.10%	111
Brij 35	0.10%	94
Tween 20	0.10%	85
Span 20	0.10%	91
Na-cholate	0.10%	96
SDS	0.05%	91
DAC	0.05%	89

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.

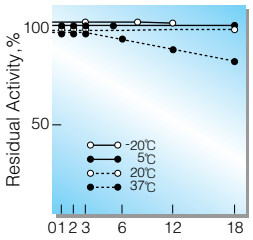

Fig.1. Stability (Powder form)

〔kept under dry conditions〕

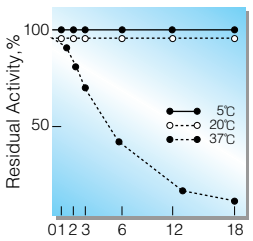

Fig.2. Stability (Liquid form)

〔enzyme concentration : 3.0U/ml
buffer composition : 0.2M borate
buffer contg. 1mM EDTA,pH8.5〕

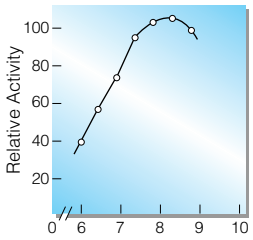

Fig.3. pH-Activity

〔25℃,in 67mM borate buffer〕

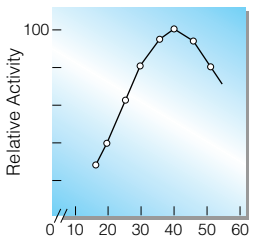

Fig.4. Temperature activity

〔5min-reaction in 50mM borate buffer, pH8.5〕

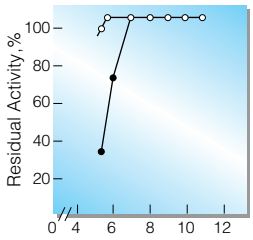

Fig.5. pH-Stability

〔25℃, 20hr-treatment with the following buffer solution: ○—○ 67mM borate ; ●—● 50mM phosphate〕

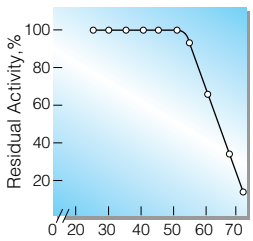

Fig.6. Thermal-stability

〔10min-treatment with 67mM borate buffer, pH8.5.〕

活性測定法（Japanese）

1.原理

Uric acid＋O_2＋2H_2O $\xrightarrow{\text{uricase}}$ Allantoin＋H_2O_2＋CO_2

尿酸の消失量を290nmにおける吸光度の変化で測定する。

2.定義

下記条件下で1分間に1マイクロモルの尿酸を酸化する酵素量を1単位(U)とする。

3.試薬

A.　0.001%　尿酸溶液〔保存溶液(0.01%)を0.001% Triton X-100及び1.0mM EDTAを含む50mMホウ酸緩衝液,pH8.5で10倍希釈する〕(用時調製)保存溶液は10.0mgの尿酸を同上緩衝液100mℓに溶解して調製する(0−5℃で保存)

B.　20%KOH溶液

酵素溶液：酵素標品を予め氷冷した0.001%Triton X-100および1.0mM EDTAを含む50mMホウ酸緩衝液,pH8.5で溶解し,同緩衝液で0.01〜0.02U/mℓに希釈し氷冷保存する。

4.手順

①試験管に下記反応混液を調製し,25℃で約5分間予備加温する。

2.0mℓ	尿酸溶液	(A)
0.5mℓ	蒸留水	

②酵素溶液0.5mℓを加え,反応を開始する。

③25℃で正確に5分間反応させた後,KOH溶液(B)0.2mℓを加えて反応を停止させる。この液につき290nmにおける吸光度を測定する(ODtest)。

④盲検は反応混液①を25℃で5分間放置後,KOH溶液(B)0.2mℓを加えて混和し,次いで酵素溶液0.5mℓを加えて調製する。以下同様に吸光度を測定する(ODblank)。

5.計算式

$$U/m\ell = \frac{\Delta OD\ (OD\ blank - OD\ test) \times 3.2(m\ell) \times 希釈倍率}{12.2 \times 1.0 \times 5(分) \times 0.5(m\ell)}$$

$$= \Delta OD \times 0.105 \times 希釈倍率$$

U/mg ＝ U/mℓ×1 / C

12.2　：　尿酸のミリモル分子吸光係数 (cm^2/micromole)

1.0　：　光路長(cm)

C　：　溶解時の酵素濃度(c mg/mℓ)