Uricase (UAO) from Candida sp.

U1493005

Uricase participates in purine catabolism. It catalyzes the conversion of highly insoluble uric acid into 5-hydroxyisourate. Accumulation of uric acid causes liver/kidney damage or chronically causes gout. In mice, a mutation in the gene encoding uricase causes a sudden increase in uric acid. Mice, deficient in this gene, exhibit hyperuricemia, hyperuricosuria, and uric acid crystalline obstructive nephropathy.

Preparation and spreparation

Appearance:White amorphous powder, lyophilized
Activity:≥4U/mg enzyme powder (containing approx.20 % of stabilizers)
Contaminants:Catalase ≤1.0 %
Stabilizers:Borate, EDTA, nonionic detergents

Storage Conditions

Store at -20℃ long term (12 months). Upon reconstitution, it is recommended to aliquot. Avoid freeze/thaw cycle.

Properties

Stability	Stable at −20℃ for at least one year	(Fig.1)
Molecular weight	approx. 120,000	-
Structure	4 subunits per enzyme molecule (Reactive SH groups are present in the enzyme molecule)	
Isoelectric point	5.4	
Michaelis constant	2.5×10^{-3}M (Uric acid)	
Inhibitors	Heavy metal ions, cyanide, various urate analogs	
Optimum pH	8.5	(Fig.4)
Optimum temperature	40℃	(Fig.5)
pH Stability	pH7.0−11.0 (25℃, 20hr)	(Fig.6)
Thermal stability	below 50℃ (pH 8.5, 10min)	(Fig.7)
Effect of various chemicals	(Table.1)	

Applications

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

Assay Principle

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

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

Unit definition

One unit causes the oxidation of one micromole of uric acid per minute under the conditions detailed 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 approximately 5 minutes.

2.0 mL Uric acid solution (A).

0.5 mL 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 0.5 mL 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 290 nm against water (OD test).

At the same time, prepare the blank by mixing the reaction mixture with 0.2 mL of KOH solution after incubation for 5 minutes at 25 ℃, and then adding the enzyme solution (OD blank).

*Dissolve the enzyme preparation in ice-cold enzyme diluent (C) and dilute to 0.01−0.02 U/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)

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

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

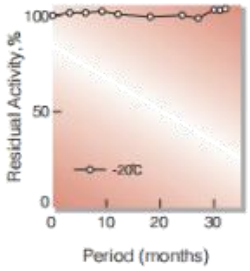

Fig.1. Stability (Powder form)

(kept under dry conditions)

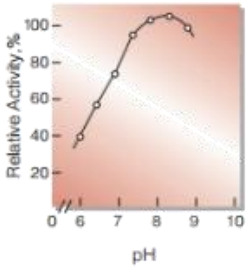

Fig.4. pH-Activity

(25℃,in 67mM borate buffer)

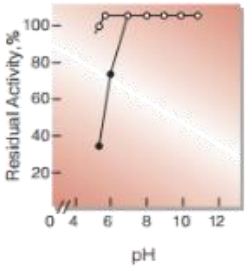

Fig.6. pH-Stability

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

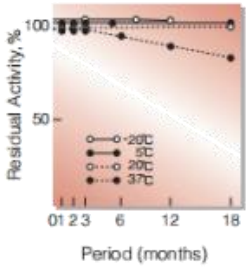

Fig.2. Stability (Powder form)

(kept under dry conditions)

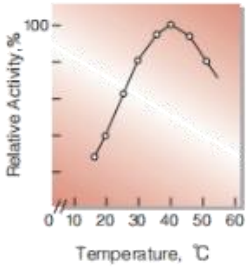

Fig.5. Temperature activity

(5min-reaction in 50mM borate buffer, pH8.5)

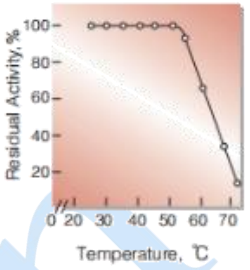

Fig.7. Thermal-stability

(10min-treatment with 67mM borate buffer, pH8.5.)

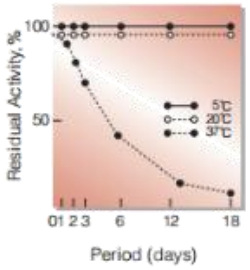

Fig.3. Stability (Liquid form)

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