Uricase (U-TE)

from recombinant *E. coli*

Urate : oxygen oxidoreductase, EC 1.7.3.3

$$\text{Urate} + H_2O + O_2 \longrightarrow \text{Unidentified products} + H_2O_2$$
$$\downarrow$$
$$CO_2 + \text{Allantoin (racemic mixture)}$$

SPECIFICATION

Appearance	light brownish lyophilizate
Activity	≧4 U/mg lyophilizate
Contaminant	catalase ≦1.0%
Stabilizer	citrate, sucrose
Storage	at −20°C

PROPERTIES

Molecular weight	*ca*. 90 kDa (gel filtration)	
Structure	2 subunits of 35 kDa (SDS-PAGE)	
Michaelis constant	1.1×10^{-5} M (uric acid)	
pH Optimum	8.5	(Fig. 1)
pH Stability	7.0–11.0	(Fig. 2)
Optimum temperature	45°C	(Fig. 3)
Thermal stability	below 55°C	(Fig. 4)
Stability (liquid form)	stable at 37°C for at least ten days	(Fig. 5)
Stability (powder form)	stable at 30°C at least three weeks	(Fig. 6)
Inhibitors	Hg^{2+}, Ag^{+}	

ASSAY PROCEDURE

Principle

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{uricase}} \text{Unidentified products} + H_2O_2$$
$$\downarrow$$
$$CO_2 + \text{Allantoin (racemic mixture)}$$

The disappearance of uric acid is measured spectrophotometrically at 290 nm.

Definition of unit

One unit (U) is defined as the amount of enzyme which oxidizes 1 μmol of uric acid per min at 25°C and pH 8.5 under the conditions described below.

Reagents

A. KOH solution, 20%: 20 g of KOH/100 ml of distilled water.
B. Uric acid solution, 0.001%: dilute the stock solution (0.01%) to 10-fold volume with enzyme dilution buffer (Reagent C) (prepare freshly). Stock solution: 10 mg of uric acid/100 ml of enzyme dilution buffer (Reagent C).
C. Enzyme dilution buffer: dissolve 3.09 g of boric acid, 0.37 g of EDTA·Na_2·$2H_2O$ and 0.01 g of Triton X-100 in 800 ml of distilled water, adjust to pH 8.5 with 2 N NaOH and dilute with distilled water to 1000 ml.

Sample: dissolve the lyophilized enzyme to a volume activity of 0.01–0.02 U/ml with ice-cold enzyme dilution buffer (Reagent C) immediately before measurement.

Procedure

1. Pipette the following reagent into a test tube.
   2.0 ml Uric acid solution (Reagent B)
   0.5 ml Distilled water
2. Equilibrate at 25°C for about 5 min.
3. Add 0.5 ml of sample and incubate for 5 min at 25°C.
4. Add 0.2 ml of KOH solution (Reagent A) to stop the reaction.
5. Read the absorbance at 290 nm in a cuvette (light path: 1 cm) (A_S).
   The blank solution is prepared by reversing the sequence of addition of sample and KOH solution (Reagent A) (A_0).

Calculation

Activity can be calculated by using the following formula:

$$\text{Volume activity (U/ml)} = \frac{(A_0 - A_S) \times 3.2\,(\text{ml}) \times df}{12.2 \times 0.5\,(\text{ml}) \times 5\,(\text{min})} = \Delta A \times 0.105 \times df$$

Weight activity (U/mg) = (U/ml) × 1/C

12.2 : Millimolar extinction coefficient of uric acid under the assay conditions ($cm^2/\mu mol$)
df : Dilution factor
C : Content of uricase preparation in sample (mg/ml)

APPLICATIONS

The enzyme is useful for the determination of uric acid in clinical analysis.

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{uricase}} \text{Unidentified products} + H_2O_2$$
$$\downarrow$$
$$CO_2 + \text{Allantoin (racemic mixture)}$$

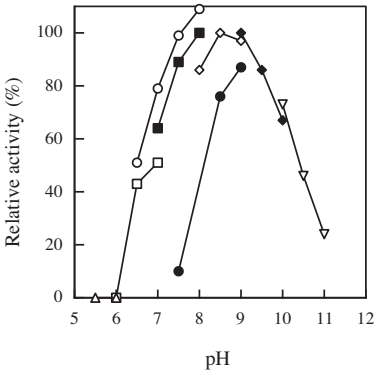
$$2\,H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + 4\,H_2O$$

REFERENCE

Koyama, Y. *et al*., *J. Biochem*., **120**, 969–973 (1996).

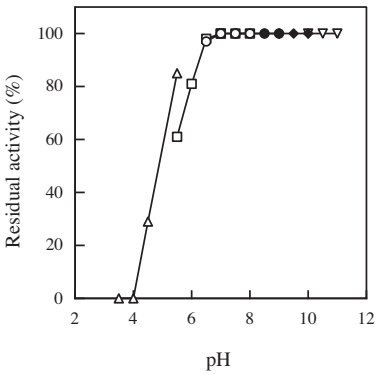
EXPERIMENTAL DATA

Fig. 1 pH Optimum



△: 50 mM acetate buffer
○: 50 mM phosphate buffer
●: 50 mM Tris–HCl buffer
□: 50 mM MES–NaOH buffer
■: 50 mM HEPES–NaOH buffer
◇: 50 mM TAPS–NaOH buffer
◆: 50 mM CHES–NaOH buffer
▽: 50 mM CAPS–NaOH buffer

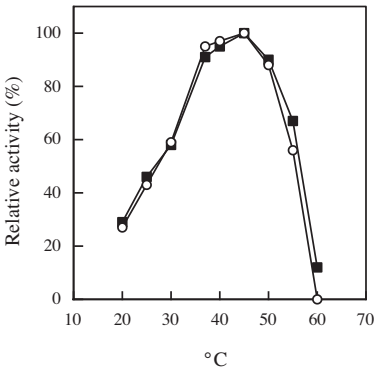
Fig. 2 pH Stability



Treatment: 25°C, 16 h

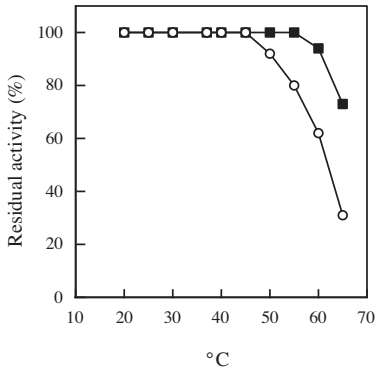
△: 50 mM acetate buffer
○: 50 mM phosphate buffer
●: 50 mM Tris–HCl buffer
□: 50 mM MES–NaOH buffer
■: 50 mM HEPES–NaOH buffer
◇: 50 mM TAPS–NaOH buffer
◆: 50 mM CHES–NaOH buffer
▽: 50 mM CAPS–NaOH buffer

Fig. 3 Optimum temperature



○: 50mM MES–NaOH buffer, pH 7.0
■: 50mM HEPES–NaOH buffer, pH 7.5

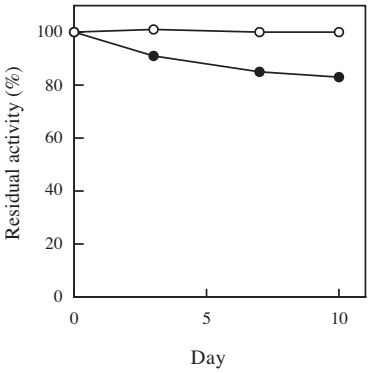
Fig. 4 Thermal stability



Treatment: 20min

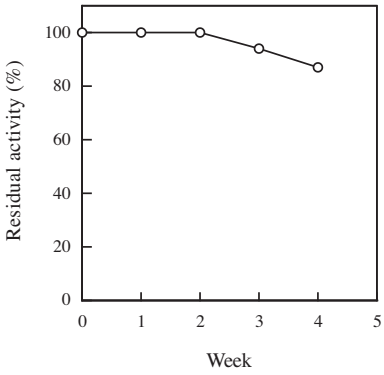
○: 50 mM MES–NaOH buffer, pH 7.0
■: 50 mM HEPES-NaOH buffer, pH 7.5

Fig. 5 Stability (liquid form) at 37°C



Kept in 10 mM MES–NaOH buffer, pH 6.8, containing 1% citrate (○), and in 10 mM MES–NaOH buffer, pH 6.8 (●)

Fig. 6 Stability (powder form) at 30°C



(Kept under dry conditions)