Uricase“Amano”3

(Urate : oxygen oxidoreductase, EC 1.7.3.3)

Uricase“Amano”3 is a uricase preparation, manufactured using recombinant bacteria.

Catalysis

Uric acid + O_2 + $2H_2O$ ⟶ Allantoin + H_2O_2 + CO_2

Specification and Preparation

Activity:	Uricase activity	≧ 2.5 u/mg (Amano method)
Appearance:	White to light yellowish brown powder, lyophilized	
Additive:	Sodium tetraborate decahydrate	

Characteristics

1. Molecular weight: 120,000 (Gel filtration)
2. Isoelectric point: 5.4
3. Km: 5.9×10^{-6} M (Uric acid)
4. Optimum pH: 8.0
5. pH stability: 7.0-10.0 (25°C, 18hrs)
6. Optimum temperature: 40°C
7. Thermal stability: up to 50°C (pH 8.5, 10 min)
8. Application: Used for the enzymatic determination of uric acid in serum in clinical diagnosis.
9. Note: Product include sodium tetraborate decahydrate which is included in the candidate of Substances of Very High Concern (SVHC) of Regulation (EC) No. 1907/2006 (REACH).

Expiration (Storage)

36 months from the date of analysis when stored at -20°C or below in a dry place under sealed conditions.

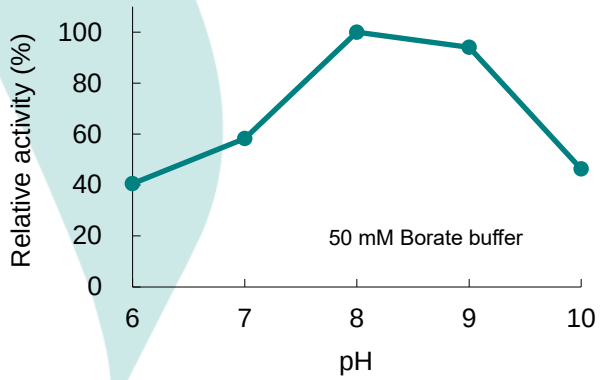
Safe Handling

1. Do not inhale.
2. In case of direct contact with skin or eyes, immediately wash or rinse with plenty of water.
3. Please refer to SDS for more details.

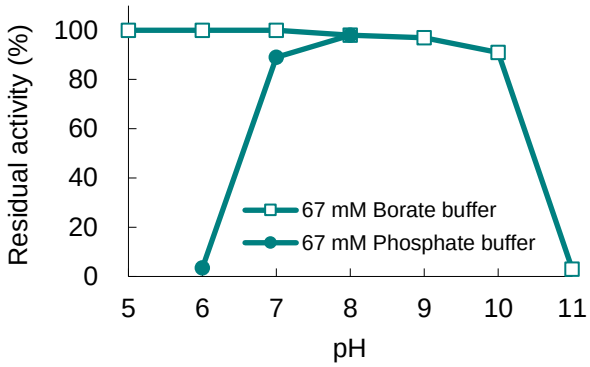
General properties

The following results demonstrate the activities of enzyme solution prepared in various buffers. Enzyme activity may vary under different experimental conditions.

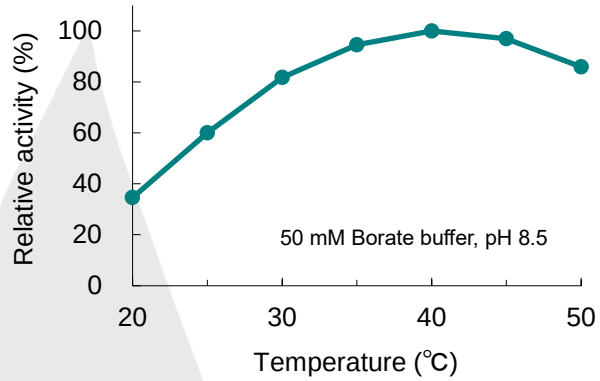
pH and Activity



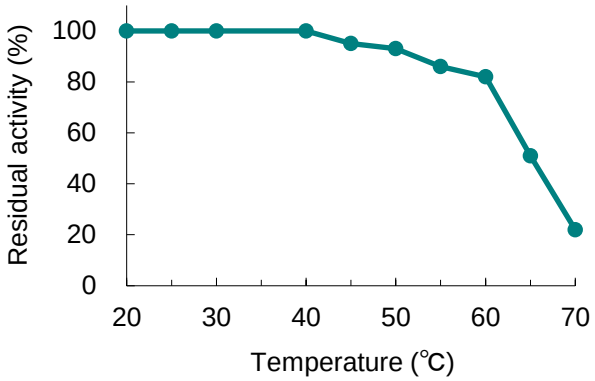
pH stability



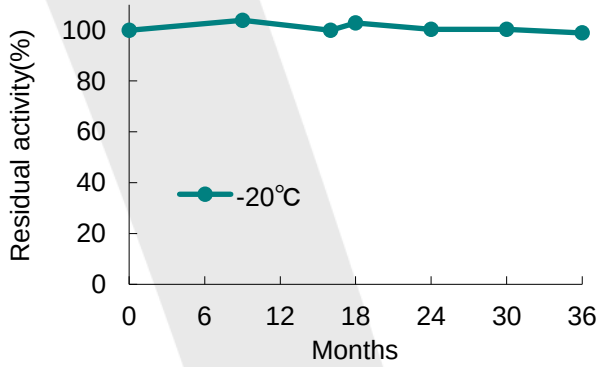
Temperature and Activity



Thermostability



Stability (powder form)



Assay method of Uricase activity

Principle

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

The consumption of Uric acid is measured at 293 nm by spectrophotometer.

Unit Definition

One unit is defined as the enzyme quantity which oxidizes one μ mole of uric acid per minute under the conditions described below.

Reagents

A. 50 mM Borate Buffer (pH 8.5)
Weigh 1.55 g of boric acid and dissolve in 400 ml of deionized water. Adjust the pH to 8.5 with 4M NaOH and fill up to 500 ml with deionized water.

B. Uric acid stock solution
Weigh 21 mg of uric acid and dissolve in approx. 50 ml of 50 mM Borate Buffer (A). Fill up to 100 ml with 50 mM Borate Buffer (A). (Can be used for 1 week if kept refrigerated)

C. Substrate solution
Dilute 5 ml of Uric acid stock solution (B) with 50 mM Borate buffer (A) and fill up to 50 ml. Keep refrigerated. (Make a fresh solution for each use)

D. Diluent
Weigh 1.36 g of potassium dihydrogen phosphate and 0.5 g of bovine serum albumin, and dissolve in approx. 80 ml of deionized water. Adjust the pH to 7.5 with 4N NaOH and fill up to 100 ml with deionized water. Keep refrigerated.

E. Enzyme solution
Weigh out Uricase“Amano”3 and dissolve in Diluent (D). Enzyme solution should be prepared so that the value of ΔOD/minute becomes in the range of 0.085±0.008.

Procedure

Pipette 3 ml of Substrate solution (C) into a quartz cell (d=10mm) and keep at 25±0.5℃ for 10 minutes. Add 0.02 mL of Enzyme solution (E) into the quartz cell and mix well. Keep the reaction mixture at 25±0.5℃. Exactly at 2 minutes and 5 minutes after the addition of Enzyme Solution (E), measure the absorbance of the reaction mixture at 293 nm. (A2 and A5)

Calculation

$$\text{Uricase activity (u/mg)} = \frac{(A2 - A5)}{3} \times \frac{1}{12.6} \times 3.02 \times \frac{n}{0.02}$$

3: Reaction time
12.6: Millimolar absorption coefficient of uric acid
3.02: Volume of the reaction mixture
0.02: Volume of Enzyme solution
n: Dilution factor of Enzyme solution

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