Uricase (UA)

U774062

Storage: -20℃. Avoid freeze/thaw cycle. Store in the dark. Desiccated.

Introduction:

Application: For the R&D and large-scale preparation of uric acid reagents.

Enzymatic Properties:

Source:	Microorganism	
Classification:	EC 1.7.3.3	
Molecular Weight:	40 kDa (SDS-PAGE)	
Isoelectric Point (pI):	5.1	
Km Value:	2.1 × 10^{-5} M (Urate)	
Inhibitors:	Fe^{3+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+}	
Optimal pH:	8.5	Fig.1
Optimal Temperature:	37℃	Fig.2
pH Stability:	5.5-10.0 (25℃, 16 h)	Fig.3
Thermal Stability:	Stable below 60℃ (pH 8.5, 30 min)	Fig.4
Storage Stability:	≥80% activity retained after 12 months of static storage at -25~-15℃	Fig.5
Protectants:	Borate, EDTA, and non-ionic surfactants	

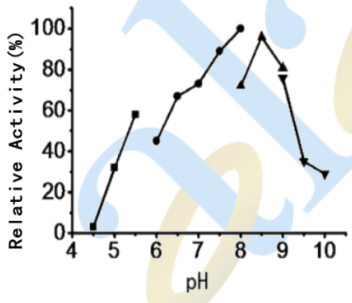


Fig. 1 Optimal pH

Buffer solution: pH 4.5-5.5, NaAc-HAc; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10.0, Glycine-NaOH.
Enzyme concentration: 1 mg/mL

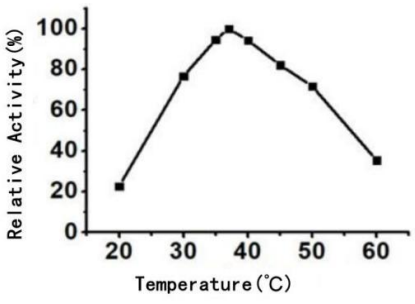


Fig. 2 Optimal Temperature

Reaction in 50 mM Boric acid buffer pH 8.5.
Enzyme concentration: 1mg/mL

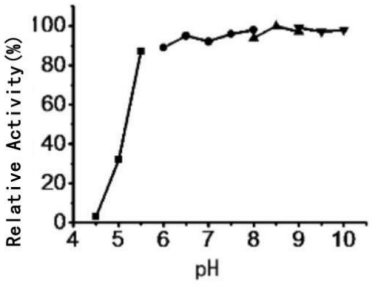


Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, NaAc-HAc; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl; pH 9.0-10, Glycine-NaOH. Enzyme concentration: 1mg/mL

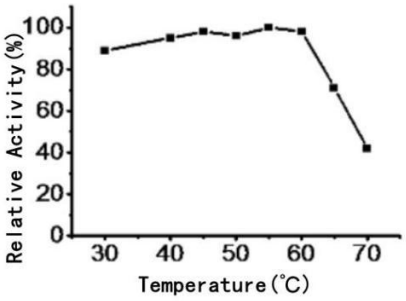


Fig. 4 Thermal Stability

30min-treatment with 50 mM Boric acid buffer, pH 8.5. Enzyme concentration: 1mg/mL

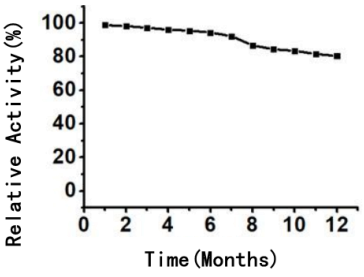
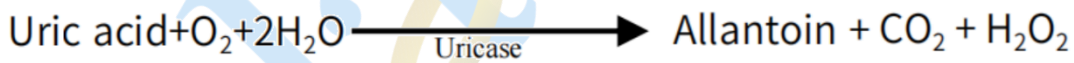


Fig. 5 Storage Stability (-25~-15℃)

Activity Assay Method:

1. Principle:

The concentration of uric acid can be determined by spectrophotometry at 290 nm.



2. Definition of Enzyme Activity:

One unit (U) of enzyme activity is defined as the amount of enzyme required to oxidize 1 μmol of uric acid per minute under the specified reaction conditions.

3. Reagent Preparation:

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 double-distilled water. Adjust the pH to 8.5 with NaOH, then bring the final volume to 1000 mL with double-distilled water.

Reagent I: 0.01% uric acid stock solution (weigh 0.01 g of uric acid, dissolve and dilute to 100 mL with Enzyme Dilution Buffer).

Reagent II: 0.001% uric acid working solution (take 10 mL of Reagent I, dilute to 100 mL with Enzyme Dilution Buffer).

Test Sample: Dilute the enzyme solution to 0.25-0.50 U/mL with Enzyme Dilution Buffer.

4. Procedure:

4.1. Preincubate Reagent II in a 37℃ water bath.

4.2. Add 2 mL of Reagent II and 0.5 mL of double-distilled water to a 3 mL cuvette. Then add 0.5 mL of the test sample and mix thoroughly.

4.3. Incbate the reaction mixture at 37℃, and measure the absorbance change (ΔAs) of the sample at 290 nm within 1 minute using a spectrophotometer.

4.4. Blank Control: Replace the enzyme solution with Enzyme Dilution Buffer and follow the same steps to obtain the blank absorbance change (ΔAb).

ΔA = ΔAs - ΔAb

Activity Calculation:

$$\text{Volume activity (U/mL)} = \frac{\Delta A \times 3.0 \times df \times 5.0}{12.2 \times 0.5 \times 1} = \Delta A \times 0.492 \times df \times 5.0$$

$$\text{Weight activity (U/mg)} = \text{Volume activity} \times 1/C$$

- 3.0: Total volume of the reaction mixture (mL).
- 0.5: Volume of the enzyme solution added (mL).
- 1.0: Light path length (cm).
- df: Dilution factor of the enzyme solution.
- C: Concentration of the enzyme solution (mg/mL).
- 5.0: Activity conversion factor.
- 12.2: Molar extinction coefficient of uric acid at 290 nm (cm²/μmol).