Uricase Activity Assay Kit

Note: The reagents are subject to change, please note and follow these instructions closely.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4435

Size:100T/48S

Components:

Extract Solution: Liquid 60 mL×1, storage at 2-8℃.

Reagent I: Liquid 5 mL×1, storage at 2-8℃.

Reagent II: Liquid 4 mL×1, storage at -20℃.

Reagent III: Liquid 10 mL×1, storage at 2-8℃.

Reagent IV: Liquid 6 mL×1, storage at 2-8℃.

Standard: Liquid 102 μL×1, storage at 2-8℃ and protect from light. Add 898 μL of distilled water to 1 mmol/mL hydrogen peroxide standard solution when the solution will be used. Reagent can be stored for 4 weeks at 2-8°C.

Preparation of working solution A: Prepared according to the sample volume in the ratio Reagent II: Reagent III: Reagent IV = 0.85mL: 2.55mL: 1.7mL (total 5.1mL, 30S). The working solution A is used for the assay of sample tubes, blank tubes and standard tubes. Prepare before use and then use. It is recommended to use within 2 hours after preparation (2-8°C or on ice).

Preparation of working solution B: Prepared according to the sample volume in the ratio Reagent II: Reagent III: Reagent I = 0.85mL: 2.55mL: 1.7mL (total 5.1mL, 30S). Working Solution B is used for the sample control tubes. Prepare before use and then use. It is recommended to use up within 2 hours after preparation (stored at 2-8°C or on ice).

Description:

Uricase, also known as uric acid oxidase, is an oxidase that participates in the purine degradation pathway. It can break down uric acid into allantoin and excrete it. Uric acid is the end product of purine metabolism. Excessive accumulation will lead to a variety of diseases such as ventilation, kidney disease and cardiovascular disease. Uricase is of great significance in the clinical detection and treatment of uric acid-related diseases.

Uricase catalyzes the decomposition of uric acid into allantoin, CO_2 and H_2O_2. H_2O_2 oxidizes Fe^{2+} in potassium ferrocyanide to form Fe^{3+}. Fe^{3+} further reacts with 4-aminoantipyrine and phenol to form red quinones, which has a characteristic absorption peak at 505 nm and reflects the activity of urase by measuring the absorbance at 505 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, low temperature centrifuge, adjustable transferpettor, water bath / constant temperature incubator, micro glass cuvettes/96 well plates, mortar/homogenizer/cell ultrasonicator , ice and distilled water.

Protocol:

I. Sample extraction:

1. Tissue:

Accordance the ratio of tissue(g) : extract solution volume (mL)=1: 5~10 (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Bacteria or cells:

Accordance the ratio of cells amount(104) : extract solution volume (mL)=500~1000: 1 (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 200w, ultrosonic 3s, interval 7s for 5 minutes). Centrifuge at 10000 rpm for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

II. Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, spectrophotometer adjust wavelength to 505 nm, set zero with distilled water.

2. Dilute the 1 mmol/mL standard solution with distilled water to a 0.5 μmol/mL standard solution and set aside. Dilution of standard solution: take 20 μL of 1 mmol/mL hydrogen peroxide standard solution, add 1980 μL of distilled water, mix thoroughly and prepare into 10 μmol/mL standard solution, then take 50 μL of 10 μmol/mL hydrogen peroxide standard solution, add 950 μL of distilled water, mix thoroughly and prepare into 0.5 μmol/mL standard solution for use, ready to use. (In the experiment, 30 μL was needed for each tube, so a large volume was prepared to reduce the experimental error).

3. Add reagents with the following list:

Reagent (μL)	Contrast tube (C)	Test tube (T)	Standard tube (St)	Blank tube (B)
Sample	30	30	-	-
Standard solution	-	-	30	-
Distilled water	-	-	-	30
Working solution A	-	170	170	170
Working solution B	170	-	-	-
Mix well, react in water bath at 37°C(mammal) or 25°C (other species) for 30 minutes. Determine the absorption value at 505 nm, record as A_C, A_T, A_{St} and A_B. Calculate $\Delta A=(A_T - A_C)$, $\Delta A_S=A_S-A_B$. Note: A control tube is required for each test tube. Testing of the same batch of samples, the soilless tube only need to be measured once or twice.				

III. Calculation of uricase activity:

1. Calculation by fresh weight of sample

Definition of unit: One unit is defined as an enzyme activity that catalyze the hydrolyze of uric acid to produce 1 μmol of H_2O_2 at pH8.8 per gram of tissue per hour.

Uricase activity(U/g weight)= $\Delta A \div (\Delta A_S \div C_S) \times V_S \div (W \times V_S \div V_E) \div T = \Delta A \div \Delta A_S \div W$

2. Calculation according to protein content

Definition of unit: One unit is defined as an enzyme activity that protein catalyze the hydrolyze of uric acid to produce 1 μmol of H_2O_2 at pH8.8 per milligram of tissue per hour.

Uricase activity(U/ mg prot)= $\Delta A \div (\Delta A_S \div C_S) \times V_S \div (Cpr \times V_S) \div T = \Delta A \div \Delta A_t \div Cpr$

3. Calculation according to cells or bacteria

Definition of unit: One unit is defined as an enzyme activity that or bacteria catalyze the hydrolyze of 1 mg of starch at pH8.8 per 1 0000 cells per hour.

Uricase activity(U/10^4 cell)= $\Delta A \div (\Delta A_S \div C_S) \times V_S \div (N \times V_S \div V_E) \div T = \Delta A \div \Delta A_S \div N$

C_S : Concentration of standard solution, 0.5 μmol/mL;

Vs: Sample volume, 0.03 mL;

V_E: Extract volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

N: The number of cells or bacteria, 10 thousand for one unit

T: Reaction time , 0.5 hour.

Note:

1. If A>1.5, please dilute the sample to appropriate concentration, multiply dilute times in the formular.

2. Working solution A and working solution B should be prepared according to the sample size, and it is recommended to use up within 2 hours. The working solution is pale yellow, and will change from pale yellow to pink, red, or even wine red over time. If discolored, it is considered invalid and needs reconfiguration.

Experimental Examples:

1. Take 0.1g of mouse liver, process the sample, take the supernatant and diluted 4 times, carry out the determination according to the operation steps. The calculation is: ΔA=At-Ac=0.804-0.285=0.519， ΔAs=As-Ab=0.741-0.051=0.690, calculate the enzyme activity according to sample weight:

Uricase Activity（U/g weight）= ΔA÷ΔAs÷W×4（diluted times）= 0.519÷0.690÷0.1×4=30.09 U/g weight

Related Products:

BC4070/BC4075	Tannase Activity Assay Kit
BC4080/BC4085	Cinnamic acid 4-hydroxylase(C4H) Activity Assay Kit
BC4090/BC4095	Anthocyanidin Reductase Activity Assay Kit
BC4100/BC4105	Indoleacetic acid oxidase Activity Assay Kit
BC4340/BC4345	Hephaestin(HP) Activity Assay Kit